PDL

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 50, Art. 6. Pages 509–678

Editor
ROY WALDO MINER

Associate Editor
PAUL LENIHAN

THE ADRENAL CORTEX

BY

R. Gaunt, G. E. Bergner, J. S. L. Browne, H. W. Deane,
R. I. Dorfman, W. J. Eversole, P. H. Forsham, Roy
O. Greep, A. G. Hills, D. J. Ingle, E. C. Kendall,
E. J. Kepler, L. A. Lewis, I. H. Page, G. Pincus,
F. T. G. Prunty, G. Sayers, M. A. Sayers,
G. W. Thorn, E. H. Venning, and
G. W. Woolley

NEW YORK
PUBLISHED BY THE ACADEMY
June 27, 1949

THE NEW YORK ACADEMY OF SCIENCES

(Lyceum of Natural History, 1817–1876)

OFFICERS, 1949

President
VICTOR K. LaMER

Vice-Presidents
GEORGE B. PEGRAM   JOSEPH C. HINSEY

Recording Secretary
HARRY A. CHARIPPER

Corresponding Secretary
RALPH H. CHENEY

Treasurer
ROBERT F. LIGHT

Editor
ROY WALDO MINER

Elected Councilors

1947–1949
WILLIAM BROWN BELL   CARYL P. HASKINS

1948–1950
ROSS F. NIGRELLI   EDWARD B. GRESSER

1949–1951
WALTER ROOT   JOHN TEE VAN   W. G. BYWATER

Finance Committee
ADDISON WEBB, *Chairman*   JOSEPH W. BARKER   GORDON BILLARD

Executive Director
EUNICE THOMAS MINER

OFFICERS OF SECTIONS

GEOLOGY AND MINERALOGY
MAURICE EWING, *Chairman*   GEORGE F. ADAMS, *Secretary*

BIOLOGY
CHARLES R. SCHROEDER, *Chairman*   ELI D. GOLDSMITH, *Secretary*

DIVISION OF MYCOLOGY
FREDERICK REISS, *Chairman*   ROYAL MONTGOMERY, *Secretary*

PSYCHOLOGY
CLAIRETTE P. ARMSTRONG, *Chairman*   LAWRENCE E. ABT, *Secretary*

ANTHROPOLOGY
JUNIUS B. BIRD, *Chairman*   JANE BELO, *Secretary*

PHYSICS AND CHEMISTRY
LEO SHEDLOVSKY, *Chairman*   TURNER ALFREY, JR., *Secretary*

OCEANOGRAPHY AND METEOROLOGY
HANS PANOFSKY, *Chairman*   JOHN C. ARMSTRONG, *Secretary*

The Sections of Geology and Mineralogy, Biology, Psychology, and Anthropology meet in regular session on Monday evenings, the Section of Physics and Chemistry on the first Tuesday, and the Division of Mycology on the fourth Friday of each month, at 8:00 o'clock from October to May, inclusive. The various Sections also hold two-day conferences at irregular periods. All meetings of the Academy are held at The American Museum of Natural History, Central Park West at 79th Street, New York 24, N. Y.

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES
Volume 50, Art 6. Pages 509–678
June 27, 1949

THE ADRENAL CORTEX*

Consulting Editor: Robert Gaunt

CONTENTS

Notes on the History of the Adrenal Cortical Problem. *By* ROBERT GAUNT AND W. J. EVERSOLE 511

The Pituitary-Adrenal System. *By* GEORGE SAYERS AND MARION A. SAYERS 522

The Chemistry and Partial Synthesis of Adrenal Steroids. *By* EDWARD C. KENDALL 540

Appendix:

(1) Studies on the Protective Power of Adrenal Extract and Steriods Against Bacterial Toxins in Adrenalectomized Rats. *By* L. A. LEWIS AND I. H. PAGE 547

(2) The Comparative Activities of 11-Dehydrocorticosterone Isolated from the Adrenal Gland and that Produced Synthetically. *By* R. I. DORFMAN 551

(3) Biological Activity of Synthetic 11-Dehydrocorticosterone Acetate. *By* E. H. VENNING 553

The Bioassay of Adrenal Cortical Steroids. *By* RALPH I. DORFMAN 556

Some Studies on the Role of the Adrenal Cortex in Organic Metabolism. *By* DWIGHT J. INGLE 576

The Cytology and Cytochemistry of the Adrenal Cortex. *By* ROY O. GREEP AND HELEN WENDLER DEANE 596

The Adrenal Cortex and its Tumors. *By* GEORGE W. WOOLLEY 616

Urinary Excretion of Adrenal Cortical Steroids. *By* ELEANOR H. VENNING AND J. S. L. BROWNE 627

Adrenal Cortex Function in Stress. *By* GREGORY PINCUS 635

Clinical Studies in Addison's Disease. *By* GEORGE W. THORN, PETER H. FORSHAM, F. T. GARNET PRUNTY, GRACE E. BERGNER, AND A. GORMAN HILLS 646

Cushing's Disease: A Primary Disorder of the Adrenal Cortices? *By* EDWIN J. KEPLER 657

---

* This series of papers is the result of a Conference on *The Adrenal Cortex*, held jointly by The New York Academy of Sciences and the New York Academy of Medicine, March 21 and 22, 1948. Publication made possible by a contribution from the General Funds of the New York Academy of Sciences.

Copyright, 1949, by The New York Academy of Sciences

NOTES ON THE HISTORY OF THE ADRENAL CORTICAL PROBLEM

By Robert Gaunt and W. J. Eversole
Department of Zoology, Syracuse University, Syracuse, N. Y.

Papers presented in symposia of this sort are prepared primarily by and for experts in the field, and are, therefore, concerned largely with new and specialized aspects of the subject. The authors of these notes, whose lot it was to arrange the program, have tried to present here a general historical introduction to the adrenal cortical problem. This was done with the hope that it might be helpful to those who have not had occasion to follow the rapidly changing currents of thought concerning adrenal function. The word "Notes" is used in the title advisedly. No attempt is made to be comprehensive, and bibliographic citation is limited largely to reviews and typical papers. We have tried to include brief mention of some of the important problems not covered elsewhere in this conference.

The progress of fruitful adrenal research in earlier years was as slow as it has now become rapid. In 1855, Thomas Addison[2] described the syndrome which bears his name, and attributed a life-maintaining function to the adrenal cortex. Brown-Sequard[10] the next year performed the first experimental adrenalectomies and verified the idea that the adrenals were essential for life. He even found, and thereby unfortunately reduced the credence that can be given this pioneer work, that the removal of one adrenal was sometimes fatal. His findings were quickly challenged by several workers,[58, 26] using mainly rats, who said that adrenal ablation did not necessarily cause death. During the remainder of the 19th century only sporadic work was done, but by about the end of the century, due to work such as that of Abelous and Langlois[1] and Boinet,[7] it was generally agreed that the adrenals were essential for life, and that apparent exceptions were due to varying amounts of accessory adrenal tissue in different species. The history of the subject until 1913 is well recorded in Biedl's monograph.[6]

An additional two decades or more were spent on the question of whether it was the adrenal cortex or medulla that was essential for life. This was largely because the discovery of epinephrine about 1900 diverted attention from any other possible adrenal secretion. Work such as that of Wheeler and Vincent[84] should have clinched the point, but acceptance of the idea was slow, and as late as 1927 Zwemer[89] initiated his studies on the adrenal with a paper showing that the cortex was the life-maintaining portion.

Adrenal Physiology of the 1920's

In a *Physiological Reviews* paper of 1924, Stewart[71] wrote, "The cortex is the part of the adrenal essential to life. How it exercises its function is utterly unknown." He raised some question as to whether its essential role was due to an internal secretion. Six years later in the same journal, Britton[8] summarized an enormous amount of information from 311 publications, but was forced to the conclusion, "That the very meagre knowledge of corticoadrenal function does not lend itself at present time even to rational theorizing." Adrenal physiology got off to an unfortunate start, or rather, largely failed to start at all until the later '20s, because the inexpert animal surgery and care usually resulted (except in rats) in such quick death after adrenalectomy that only animals in a state of acute collapse were available for experimental study.

The perpetrators of poor surgery were eloquently chastised by Stewart[71] who, among his other contributions, left the brightest prose in the adrenal record. The insistence by Stewart and Rogoff on a high standard of surgical technique and animal care in adrenal work, and their demonstration that when such rules were practiced adrenalectomized dogs lived for periods of approximately 10 days,[64] rather than the shorter periods generally found before, was a contribution of great worth in inaugurating sound physiological studies.

As one looks back with the benefit of hindsight at the information on the adrenal cortex accumulated prior to 1930, the abjectly negative conclusions of the above-mentioned reviewers seem overdrawn. The lack, of course, was the key facts to which their available information could be related—among which was the lack of definite proof of the existence of an adrenal cortical hormone.

Placed in context at the present time, pre-1930 statements such as the following have a modern ring. It was, for instance, reported that there was a rise in the R.B.C. count in adrenal insufficiency,[23] an increase in viscosity and concentration of the blood,[38, 49] with a rise in urea[16, 59, 51] and in protein and total N,[74] possibly due to an increased susceptibility to histamine.[13] Some workers specifically associated these changes with an altered kidney function,[16, 51] and the existence of a low blood volume was recognized.[38, 88]

It was found that blood chlorides decreased after adrenalectomy,[49, 63] as did Na, pH, CO_2 tension and serum bicarbonate,[75] while total solids, K and Mg rose.[5] The administration of Ringer's solution was found to be beneficial to adrenalectomized animals[72] and the salutary effects attributed largely to Na salts.[50]

The possible relation of the adrenal cortex to cholesterol metabolism was extensively considered without conclusive results.[61]

Many workers found a low blood sugar at some stage of adrenal insufficiency, and it was known that liver glycogen levels were affected by adrenalectomy,[51, 8, 80] although there were slight if any changes in muscle glycogen.[12] The mechanisms involved were not known, and the distinction between cortical and medullary effects were sometimes vague.

Adrenalectomized animals were known to be susceptible to a host of stresses, drugs, toxins, etc.,[80, 82] but the tendency was more to try to interpret each susceptibility individually, rather than as expressions of a single mechanism as is now done.[82, 69]

The relative enlargement of the lymphatic tissues[4] and the increase in lymphocytes[90] after adrenalectomy were known. The importance of such observations as they relate to the resistance mechanisms of the body have, however, only been recently clarified.[85]

The relation of the adrenal to the adrenogenital syndrome excited great interest,[22, 35] and when cortical extracts were available attempts were immediately made to identify the "virilizing hormone." There was little initial success, but later the elaboration of androgenic steroids by the adrenal was established.[87] Stewart and Rogoff[65] had first shown that some influence, later identified as progesterone,[21] was present during pregnancy and after estrus in the dog, which prevented the development of adrenal insufficiency.

None of this work, however, was such as to permit a definite and convincing statement as to the function of the adrenal cortex. As the work accumulated, the symptoms of adrenal insufficiency became legion; obviously, few functions or organs were unaffected by adrenalectomy. In not a few cases, convictions and the language used to express them, were strong, even if fact and theory were shaky. Like Henry Ward Beecher's ministerial friend, the early adrenal workers were not without recourse to the use of shouts to overcome doubts. The bellicosity carried over into the '30s and enlivened physiological circles of that era. It was reminiscent of the verbal mayhem that troubled the pages of the American Journal of Physiology as the story of the function of the adrenal medulla evolved a few years earlier.

Adrenal Physiology after 1930

In 1930, the modern history of the adrenal cortex was opened. In that year, the preparation of the first good cortical extracts was announced. Final proof of the endocrine nature of adrenal cortex function was thereby afforded, and a completely new approach to physiological and clinical problems made available.

The status of the subject by 1937 is well summarized in the papers of the Cold Spring Harbor Symposium of that year.[81] Since then, a variety of reviews have treated general or specific aspects of the subject,[82, 73, 39, 57, 62, 36, 40, 55] that of Swingle and Remington[80] being the most comprehensive.

Extraction of Hormones of the Adrenal Cortex. Many investigators, over a long period of time, attempted to extract the cortical hormones. The problem was difficult for various reasons, among which was the low concentration of hormone in the gland and the difficulty of eliminating epinephrine as a contaminant. How credit should be divided for the success of these early efforts is, perhaps, a matter of opinion. Weakly active preparations may have been obtained before 1930.[8] It is generally agreed that Swingle and Pfiffner,[77, 78] and Hartman, Brownell, *et al.* [33, 31] first described methods based on lipoid extraction procedures which proved to produce extracts that would keep adrenalectomized animals and Addisonian patients alive indefinitely. The Swingle-Pfiffner preparations were the ones most widely used. Hartman termed the active principle of the extracts "cortin."

Swingle and Pfiffner, in their first attempts to establish the potency of their preparations, adopted the rigorous procedure of demanding that they maintain adrenalectomized cats for 100 days. The extracts were weak by modern standards, and the use of about 5 cc. per day was necessary. They were also expensive, costing about a dollar per cc. to prepare. These 100-day cats, in which roughly five hundred dollars had been injected, were viewed with some economic misgiving by those of us who, as graduate students in the Princeton laboratories at the time, were not sharing in the alleged prosperity of early 1929.

The first clinical trial with this extract in which a moribund patient was quickly revived was made in the Mayo Clinic by Drs. Rowntree and Greene.[66]

Isolation of Active Principles of Extract of the Adrenal Cortex. The physiological work of the early '30s was done with whole adrenal cortical extract, and under the assumption by most workers that it contained only one hormone. In 1936, however, work from the laboratories of Wintersteiner and Pfiffner, Reichstein, and Kendall began to show that a large number of steroids could be crystallized from cortical extract.[57, 62, 40] There was confusion at first, due to the lack of knowledge as to what to look for, concerning the nature or presence of biological activity in some of the compounds. 17-Hydroxy-11-dehydrocorticosterone, one of the potent steroids in affecting organic metabolism, was thought by Wintersteiner and Pfiffner to be inactive when they first isolated it, because of its weakness in life-maintaining activity, for which they were testing. Its biological properties were first revealed by Mason, Meyers, and Kendall,[52] using Ingle's muscle work test. Of the 28 adrenal steroids thus far isolated, only 6 are known to have corticoid activity, although some others have sex hormone activity. The latest work implies that the known active steroids exert qualitatively different effects (*cf.* Kendall's paper in this monograph). Most groups have found an amorphous fraction remaining in their extracts after known steroids have been removed which is highly active in maintaining life. Further study of the nature of this fraction is awaited with great interest.

The only adrenal steroid for which a cheap method of synthetic production has been available is desoxycorticosterone, generally used as its acetate (DCA). The small amounts of steroids in adrenal tissue preclude quantity production by extraction. Great difficulties were encountered in finding a synthetic method for the production of those steroids oxygenated at carbon-11. The recent discovery of such methods, effective if not cheap, as presented here by Dr. Kendall, is one of the most important events in adrenal history.

Desoxycorticosterone—Is It a Cortical Hormone? The biological properties of DCA have been extensively studied, and considerable physiological interpretation based upon them. Several of its properties are unique to known steroids. Reichstein is the only adrenal chemist to have obtained it from adrenal tissue, and he found it in very small quantity. There is basis for serious question as to whether this compound is secreted in significant quantities into the blood stream, and this creates the need for caution in interpreting physiological experiments based on its use.

Early Experiments with Cortical Extract. Armed with potent cortical extracts, investigators could make a fresh approach after 1930 to an analysis of adrenal function. One of the motivating hopes of the Princeton group, and probably others, in preparing such extracts was that overdosage phenomena could be produced which would reveal the essential nature of cortical function more clearly than studies of adrenal insufficiency had done. The first attempts to produce such overdosage phenomena were made in the late Dr. Harrop's laboratory at Baltimore. As much as one hundred ml. of extract were injected into dogs, and 15 blood constituents were measured.[28] No effects at all were found! This disheartening result was the more unfortunate because slight variations in their technical procedure should have revealed clues of great importance. Many overdosage effects have since been seen.

Electrolyte and Water Metabolism. By 1932, however, the work leading to an appreciation of the role of cortical hormones in electrolyte and water metabolism was under way. Attention was recalled to the similarity of adrenal insufficiency and secondary shock, and the probable importance of the declining blood volume emphasized.[79] Loeb, *et al.*,[43] followed shortly by the Harrop group,[30, 29] noted in adrenal insufficiency a marked loss of plasma Na, due to renal wastage, and thus, apparently, accounted for the extracellular dehydration. More dramatically, Loeb[42] showed that NaCl alone would maintain an Addisonian patient.

With this report, most adrenal workers rushed to their laboratories with salt shakers in hand. It was generally found that salt would not maintain adrenalectomized animals indefinitely, but all agreed it was helpful. Rubin and Krick[67] contributed the highly useful technique of adding salt to the drinking water of adrenalectomized rats. Allers and Kendall[3] perfected a diet, high in a mixture of Na salts and low in K, which would maintain adrenalectomized dogs indefinitely. It was generally found that salt-treated animals could not withstand stress well. Harrison and Darrow[27] showed that a defect in renal tubular function accounted for the loss of Na and retention of K that occurred in adrenalectomized animals. For awhile, it was considered by some that *the* function of the cortical hormone was to act on the kidney and to regulate electrolyte and water excretion.

When it became apparent that the disordered Na metabolism could in itself hardly explain all of adrenal insufficiency, attention was focussed on K, particularly by Zwemer.[91] It had been shown[5, 34] that K might rise to toxic levels in adrenalectomized animals, and important details were added by others.[80]

Adrenal insufficiency was not destined to be so simply catalogued. The alterations in Na metabolism are undoubtedly important, as shown, if in no other way, by the therapeutic value of salt administration, but animals can recover from adrenal insufficiency without replacement of the Na if enough cortical hormone is made available.[76] The accumulation of K is likewise dangerous when it occurs, but many have found that a rise of plasma K is not necessarily prerequisite to adrenal insufficiency. The regulation of K metabolism is clearly a major adrenal function, but in the complex syndrome of adrenal insufficiency many other factors are involved as well.

When crystalline cortical steroids became available, it was soon shown that DCA was the most potent in causing a retention of Na and excretion of K, either in adrenalectomized or normal animals.[80, 39, 36] Most cortical (and sex) steroids cause some Na-retention, with the exception of those oxygenated at C-17 which, transiently at least, may cause Na loss.[83, 11, 37] The sex steroids have a Na-retaining effect, but of these only progesterone is beneficial in prolonging the lives of adrenalectomized animals. The estrogens, for reasons not established, are toxic after adrenalectomy.[21]

It is doubtful now, as first thought, that the alterations in water metabolism which characterize adrenal insufficiency are entirely a passive response to the changing electrolyte levels. When DCA is given and Na retention induced, water, as might be expected, is retained as well. In the normal dog, and in hypophysectomized animals of other species, however, the chronic administration of DCA can lead to diabetes insipidus-like effects.[60, 70, 86] Cortical extract (which presumably contains, largely, the adrenal steroids oxygenated at C-11) is less effective, if active at all, in inducing this diuretic response.[68] On the other hand, a dramatic diuretic effect of cortical extract, or 17-hydroxy-11-dehydrocorticosterone, is seen if these substances are given to animals which have been hydrated by the forced feeding of water; this is to be correlated with the absence of normal water diuresis in adrenal insufficiency.[19, 41] Thus, the cortical hormones may be diuretic or anti-diuretic agents, depending upon various circumstances. A precise statement of these circumstances is not possible at present, but it is yet to be demonstrated that the alterations in water metabolism are entirely secondary to those of electrolytes.

Carbohydrate Metabolism. Early workers, as indicated before, noted changes in carbohydrate metabolism, such as low blood sugar and liver glycogen levels in adrenal insufficiency. Many workers considered these changes terminal, however, and a likely consequence of moribundity rather than an indication of a primary function of the adrenal cortex.

Dr. S. W. Britton, after 1930, began a long series of experiments leading him to a vigorous enunciation of the theory that the "prepotent function" of the adrenal cortex was to regulate carbohydrate metabolism.[9] His arguments were based primarily on two types of observations: first, that adrenalectomized animals developed acute hypoglycemia and low glycogen levels, and, second, that when normal fasted animals were given large doses of cortical extracts there was an increase in blood sugar and in both liver and muscle glycogen. Britton's story was viewed with considerable misgiving in the early '30s, partly because his interpretations were sweeping and seemed to relegate the concurrent work on electrolyte metabolism to a secondary role, and partly becauses of question raised as to some of the experimental details.[45] Most of this stormily debated work now fits into the picture of established fact when reinterpreted in light of modern knowledge of the multiple nature of cortical secretions, and modified to recognize the primary role of pituitary hormones in regulating muscle glycogen.

Long and Lukens provided the evidence resulting in widespread acceptance of the idea that in the regulation of organic metabolism the adrenal cortex had a role of direct rather than secondary importance. Their work started with the conclusive demonstration of the fact, first indicated by Hartman and Brownell,[32] that adrenalectomy ameliorated pancreatic diabetes,[47, 44] and the finding by G. Evans, in Long's laboratory, that the adrenal cortices were involved in gluconeogenic processes.[14]

It was first thought that the action of cortical hormones was primarily to stimulate gluconeogenesis. Later the factor of an inhibition of carbohydrate utilization was demonstrated,[46] and has assumed increasing importance in interpretation of later work (*e.g.*, Ingle's paper in this monograph).

As quickly as crystalline adrenal steroids were made available, it became clear from reports of remarkable unanimity that the 11-oxygenated adrenal steroids alone exerted these dramatic effects on the metabolism of carbohydrates and related substances.[80, 36] While there is, in all probability, more common ground than has yet been understood between the relation of adrenal steroids to organic and electrolyte metabolism, it was clear by 1940 that these two aspects of adrenal function were probably under the control of different adrenal steroids. Attempts to explain them in terms of the action of one hormone had resulted in much of the earlier confusion. With these basic facts established, subsequent progress along these lines has been rapid, as indicated in the paper submitted here by Dr. Ingle.

Other Aspects of Adrenal Function

Because of the historical factors traced above, it has become customary to speak of adrenal function as having two main aspects: (a) a role in electrolyte metabolism, mediated primarily by a DCA-type of steroid, and (b) a role in carbohydrate and protein metabolism, mediated by the 11-oxygenated steroids. A third is sometimes and properly added, namely: a role, as yet ill-defined, of the androgenic adrenal steroids. Obviously, disorders in any of these categories involve a host of sequela, the primary or secondary nature of which is difficult to establish, and there are a great many well-established adrenal functions whose relation to such processes is not yet clear. Some examples follow.

Stress. The relation of the adrenal to stress is the keynote of this monograph; nearly all of the papers touch on some aspect of the subject. The older literature is replete with references to the fact that adrenalectomized animals are susceptible to stresses of almost every variety: cold, toxins, trauma, drugs, infections, etc. The earlier tendency was to try to relate a specific adrenal function to each of these susceptibilities.

It is clear now from the work reviewed in this monograph by Selye, Pincus, Sayers, and others that there is a non-specific physiological response to stresses in general, resulting in the acquisition of resistance, and that one important phase of that response is adrenal hyperfunction. The adrenal stimulation is the result of an increased output of pituitary adrenocorticotrophin, and without such pituitary-adrenal activation the resistance to all stresses is low.

The mechanism of the protective action afforded by cortical hormones is unknown for the most part, but one important aspect of it has been elucidated in recent years by White, Dougherty, and their associates. They have shown that increased cortical hormone levels induced by stress act on the lymphatic system to cause a dissolution of certain of its elements and a release into the blood of gamma globulins (antibody proteins) for use by the organism. Their work was presented at an earlier Academy conference[85] and hence was not repeated here. The steroids active in protecting against stress are primarily those oxygenated at C-11.[80]

The fact that cortical hormones are involved in the resistance to stress led logically to attempts, particularly during the war, to protect normal individuals against trauma, etc., by the administration of large doses of these hormones. In other words, an attempt was made to produce a super-normal resistance.

Results of such attempts were conflicting, but the weight of the greatest number of reports lies on the side of those who found the cortical hormones of slight, if any, usefulness in producing a hyper-resistance in animals or patients whose own adrenals were intact.[80] The basis for such findings, presumably, is, that in most cases, the adrenals can put out hormone at such an elevated rate in response to stress that still higher levels applied by injection is of little avail. A further theoretically possible factor is that, unless enormous doses of cortical hormone are administered, the effect of that given will merely be to inhibit release of pituitary adrenotrophin, which in turn would cut down the rate of adrenal secretion, and hence result in no net addition to the total amounts of hormone available to the organism.

There are, however, some conditions in which the administration of cortical hormones to intact animals will provide clearly a life-maintaining protection, *e.g.*, in K intoxication,[48] in water intoxication,[18] and probably in insulin hypoglycemia.[24] It so happens that these are conditions of little clinical significance, or ones for which better therapies are available. They do indicate, however, together with much other evidence, that a search for the therapeutic usefulness of cortical hormones other than in frank Addison's disease is worth while.

Circulation. When adrenalectomized animals collapse under stress, a circulatory failure is the most conspicuous feature of the process. A role of the cortical hormones in maintaining the functional integrity of the circulatory system is clearly indicated. The mechanisms involved are not. Presumably, both the vascular musculature and capillary permeability are involved.[80]

Reproduction and Lactation. The ability of the adrenal cortices to secrete sex steroids is well established and the physiological and pathological implications have been extensively studied.[55]

A role of the cortical hormone in supporting lactation is also clearly seen,[54] and an association with the enzymology of milk production has been suggested.[15]

The Rat and the Adrenal Problem

Adrenal experimental work is now done more on rats than on any other form. Until 1930, many investigators considered the rat unsuitable for experimental use, largely because, in the hands of most workers, and presumably because of the presence of adrenal accessories, this species survived adrenalectomy in 50 per cent or more of the cases.

In 1930, Pencharz *et al.*[56] reported that the rat was no exception to the rule that adrenalectomy was fatal. This statement attracted considerable attention, because the ease and economy of doing adrenal work with a rat colony was obvious. A flurry of papers was forthcoming, most of which agreed that rats rarely survive adrenalectomy.[17] This fact, together with the development of more and better micromethods for blood work and the conquest of surgical problems such as hypophysectomy and pancreatectomy, which frequently need to be combined with adrenalectomy, has made the rat suitable for most types of adrenal study.

The factors which account for the survival or non-survival of rats after adrenalectomy, however, are still far from completely defined. Some of the high survival figures reported by early workers may have been due to the use of scrap diets high in NaCl. It was shown[20] that distinct colony differences could account for variation in survival in some cases, and this has been seen now by several workers. The opinion is widely held, on the other hand, that indefinite survivals are due to incomplete adrenal removal or to failure to remove the peri-adrenal tissue, the presumed site of accessories. This argument is not sympathetically received by those of us who think we can do this simple operation, but who still see occasional instances of prolonged survival, particularly in adult adrenalectomized rats after some sustaining therapy has been withdrawn.

More perplexing, however, is the difficulty of modern investigators in actually finding the adrenal accessories (or fragments) which are supposed to account for survival. Two possibilities seem to exist. First, it may be that the rat, one of the most rugged and adaptable mammals, can, in rare cases, maintain itself indefinitely under mild laboratory conditions without cortical hormones or unusual dietary support. These long-surviving animals cannot withstand stress well. Secondly, McFarland[53] has recently stated that, if adrenalectomized rats are maintained on salt for awhile, functional cortical masses may then differentiate from coelomic mesothelium without forming all of the typical histological adrenal layers. This is reminiscent of the fact that the ovary in the adrenalectomized ground squirrel can assume cortical functions.[25] We believe we have seen in several instances the type of tissue to which McFarland refers, but, in the absence of detailed cytological study hesitated to call it adrenal tissue. The problem is worth further study.

Conclusion

A list of the actions of cortical hormones could be continued almost indefinitely. It has been said that the ubiquitous finger of the pituitary reaches into almost all bodily processes (Evans). No less is this true of the adrenal cortex. The complete story of adrenal function could not be told in a two-day conference. It is the feeling and hope of those who arranged the program, however, that the papers presented here, while revealing a record of great accomplishment, will also presage a still more productive future.

Bibliography

1. Abelous & Langlois. 1895. C. R. Soc. Biol. **2:** 334.
2. Addison, T. 1855. *On the Constitutional and Local Effects of Disease of the Supra-renal Capsules.* London.

3. Allers, W. D., & E. C. Kendall. 1937. Am. J. Physiol. **118**: 87.
4. Banting, F. G., & S. Gairns. 1926. Am. J. Physiol. **77**: 100.
5. Baumann, E. J., & S. Kurland. 1927. J. Biol. Chem. **71**: 281.
6. Biedl, A. 1913. *Internal Secretary Organs.* William Wood & Co. New York.
7. Boinet, E. 1895. C. R. Soc. Biol. **47**: 162.
8. Britton, S. W. 1930. Physiol. Rev. **10**: 617.
9. Britton, S. W., & H. Silvette. 1937. Symp. Quant. Biol. Cold Spring **5**: 357.
10. Brown-Sequard. 1856. C. R. Acad. Sci. **43**: 422, 542.
11. Clinton, M., Jr., & G. W. Thorn. 1942. Science **96**: 343.
12. Cori, C. F., & G. T. Cori. 1927. J. Biol. Chem. **74**: 473.
13. Dale, H. H. 1929. Lancet. **1**: 1179.
14. Evans, G. 1936. Am. J. Physiol. **114**: 297.
15. Folley, S. J., & A. L. Greenbaum. 1946. Biochem. J. **40**: 46.
16. Gaillard, L. 1914. Bull. Mem. Soc. Méd. Hôp. Paris. **37**: 272.
17. Gaunt, R. 1933. Am. J. Physiol. **103**: 494.
18. Gaunt, R. 1943. Proc. Soc. Exp. Biol. & Med. **54**: 19.
19. Gaunt, Robert. 1946. J. Clin. Endocrinol. **6**: 595.
20. Gaunt, R., J. H. Gaunt, & C. E. Tobin. 1935. Proc. Soc. Exp. Biol. & Med. **32**: 888.
21. Gaunt, R., & H. W. Hays. 1938. Am. J. Physiol. **124**: 767.
22. Glynn, E. E. 1911. Quart. J. Med. **5**: 157.
23. Gradinescu, A. V. 1913. Pflüg. Arch. **152**: 187.
24. Grattan, J. F., & H. Jensen. 1940. J. B. C. **135**: 511.
25. Groat, R. A. 1943. Endocrinology. **32**: 488.
26. Harley, G. 1858. Trans. Path. Soc. Lond. **9**: 40.
27. Harrison, H. E., & D. C. Darrow. 1939. Am. J. Physiol. **125**: 631.
28. Harrop, G. A., J. J. Pfiffner, A. Weinstein, & W. W. Swingle. 1931. Science **73**: 683.
29. Harrop, G. A., L. J. Soffer, R. Ellsworth, & J. H. Trescher. 1933. J. Exp. Med. **58**: 17.
30. Harrop, G. A., A. Weinstein, L. J. Soffer, & J. H. Trescher. 1933. J. Exp. Med. **58**: 1.
31. Hartman, F. A., & K. A. Brownell. 1930. Science **72**: 76.
32. Hartman, F. A., & K. A. Brownell. 1934. Proc. Soc. Exp. Biol. & Med. **31**: 834.
33. Hartman, F. A., K. A. Brownell, & W. E. Hartman. 1930. Am. J. Physiol. **95**: 670.
34. Hastings, A. B., & E. L. Compere. 1931. Proc. Soc. Exp. Biol. & Med. **28**: 376.
35. Heally, C. E., & C. C. Guy. 1931. Arch. Path. **12**: 543.
36. Ingle, D. J. 1944. *Chemistry and Physiology of Hormones:* 83. A.A.A.S. Washington, D. C.
37. Ingle, D. J., R. Sheppard, E. A. Oberle, & M. H. Kuizenga. 1946. Endocrinology **39**: 52.
38. Kellaway, C. H., & H. S. Cowell. 1923. J. Physiol. **57**: 82.
39. Kendall, E. C. 1941. Arch. Path. **32**: 474.
40. Kuizenga, M. H. 1944. *Chemistry and Physiology of Hormones:* 57. A.A.A.S. Washington, D. C.
41. Levy, M. S., M. H. Power, & E. J. Kepler. 1946. J. Clin. Endocrinol. **6**: 607.
42. Loeb, R. F. 1933. Proc. Soc. Exp. Bio. & Med. **30**: 808.
43. Loeb, R. F., D. W. Atchley, E. M. Benedict, & J. Leland. 1933. J. Exp. Med. **57**: 775.
44. Long, C. N. H. 1937. Symp. Quant. Biol. Cold Spring Harbor **5**: 344.
45. Long, C. N. H. 1937. Symp. Quant. Biol. Cold Spring Harbor **5**: 359.
46. Long, C. N. H., B. Katzin, & E. G. Fry. 1940. Endocrinology **26**: 309.
47. Long, C. N. H., & F. D. W. Lukens. 1936. J. Exp. Med. **63**: 465.
48. Lowenstein, B. E., & R. L. Zwemer. 1943. Endocrinology **33**: 361.
49. Lucas, G. H. W. 1926. Am. J. Physiol. **77**: 114.
50. Marine, D., & E. J. Baumann. 1927. Am. J. Physiol. **81**: 86.
51. Marshall, E. K., & D. M. Davis. 1916. J. Pharm. & Exp. Therap. **8**: 525.
52. Mason, H. L., C. S. Meyers, & E. C. Kendall. 1936. J. B. C. **116**: 267.
53. McFarland, W. E. 1945. Anat. Rec. **93**: 233–249.
54. Nelson, W. O., R. Gaunt, & M. Schweizer. 1943. Endocrinology **33**: 325.
55. Parkes, A. S. 1945. Physiol. Rev. **25**: 203.
56. Pencharz, R. I., J. M. D. Olmstead, & G. Giragossintz. 1930. Science **72**: 175.
57. Pfiffner, J. J. 1942. Advances in Enzymology **2**: 325.
58. Philipeaux. 1856. C. R. Acad. Sci. **43**: 904.

59. Porak, R., & H. Chabanier. 1914. C. R. Soc. Biol. **77:** 440.
60. Ragan, C., J. W. Ferrebee, P. Phyfe, D. W. Atchley, & R. F. Loeb. 1940. Am. J. Physiol. **131:** 73.
61. Randles, F. S., & A. Knudson. 1928. J. Biol. Chem. **76:** 89.
62. Reichstein, T. 1943. *The Hormones of the Adrenal Cortex. Vitamins and Hormones.* Academic Press. New York.
63. Rogoff, J. M., & G. N. Stewart. 1926. Am. J. Physiol. **78:** 711.
64. Rogoff, J. M., & G. N. Stewart. 1928. Am. J. Physiol. **84:** 660.
65. Rogoff, J. M., & G. N. Stewart. 1928. Am. J. Physiol. **86:** 20.
66. Rowntree, L. G., C. H. Greene, W. W. Swingle, & J. J. Pfiffner. 1930. Science **72:** 482.
67. Rubin, M. I., & E. T. Krick. 1936. J. Clin. Invest. **15:** 685.
68. Schweizer, M., A. Ehrenberg, & R. Gaunt. 1943. Proc. Soc. Exp. Biol. & Med. **52:** 349.
69. Selye, Hans. 1946. J. Clin. Endocrinol. **6:** 117.
70. Selye, H., & L. Bassett. 1940. Proc. Soc. Exp. Biol. & Med. **45:** 272.
71. Stewart, G. N. 1924. Physiol. Rev. **4:** 163.
72. Stewart, G. N., & J. M. Rogoff. 1925. Proc. Soc. Exp. Biol. & Med. **22:** 394.
73. Swann, H. G. 1940. Physiol. Rev. **20:** 493.
74. Swingle, W. W. 1927. Am. J. Physiol. **79:** 666.
75. Swingle, W. W., & A. J. Eisenman. 1927. Am. J. Physiol. **79:** 679.
76. Swingle, W. W., W. M. Parkins, A. R. Taylor, & H. W. Hays. 1936. Am. J. Physiol. **116:** 438.
77. Swingle, W. W., & J. J. Pfiffner. 1929. Anat. Rec. **44:** 225.
78. Swingle, W. W., & J. J. Pfiffner. 1930. Science **71:** 321.
79. Swingle, W. W., J. J. Pfiffner, N. M. Vars, P. A. Bott, & W. M. Parkins. 1933. Science **77:** 58.
80. Swingle, W. W., & J. W. Remington. 1944. Physiol. Rev. **24:** 89.
81. Symposium. 1937. Symp. Quant. Biol. Cold Spring Harbor **5**.
82. Tepperman, J., F. L. Engel, & C. N. H. Long. 1943. Endocrinology **32:** 373.
83. Thorn, G. W., & G. A. Harrop. 1937. Science **86:** 40.
84. Wheeler, T. D., & S. Vincent. 1917. Trans. Roy. Soc. Canada **11:** 125.
85. White, A., & T. F. Dougherty. 1946. Ann. N. Y. Acad. Sci. **46:** 859.
86. Winter, C. A., & W. R. Ingram. 1943. Am. J. Physiol. **139:** 710.
87. Wintersteiner, O. 1942. Gland. Physiol. Therapy. A.M.A. **21:** 327.
88. Wyman, L. C., & C. tum Suden. 1930. Am. J. Physiol. **94:** 579.
89. Zwemer, R. L. 1927. Am. J. Physiol. **79:** 641.
90. Zwemer, R. L., & C. Lyons. 1928. Am. J. Physiol. **76:** 545.
91. Zwemer, R. L., & R. Truszkowski. 1937. Endocrinology **21:** 40.

THE PITUITARY-ADRENAL SYSTEM

By George Sayers and Marion A. Sayers

Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah

The fluctuating needs of the tissue cells of the organism for cortical hormone, as it adjusts to environmental change or resists stress, are met by the combined activities of the pituitary and the adrenal cortex. It is convenient to consider the peripheral tissue cells, the pituitary, and the adrenal cortex as interdependent members of a system, the pituitary-adrenal system. Stress, applied to the peripheral tissue cells, results in activation of the anterior pituitary. The anterior pituitary in turn accelerates the secretory activity of the adrenal cortex through the elaboration of adrenocorticotrophic hormone (A.C.T.H.).

The first part of this paper will consider manifestations of adrenal cortical activity associated with a variety of environmental changes and stressful conditions. The second part will consider the mechanism whereby the great variety of non-specific stresses brings about an increase in the rate of elaboration of A.C.T.H. from the pituitary.

*Indices and Classification of Adrenal Cortical Activity**

With the availability of purified A.C.T.H.,[35, 64] it soon became evident that all of the morphological and chemical changes in the adrenal cortex which had been described as taking place following subjection of the animal to stress could be reproduced by the administration of this trophic substance.

A.C.T.H.,[63] as well as noxious stimuli,[61, 69] increases the gross size of the adrenal gland.

The sudanophilic substance, which most likely is cholesterol ester material, undergoes the same type of dynamic change following administration of A.C.T.H. as it does following subjection of the animal to acute stress.[61] These studies have made it possible to correlate the concentration of sudanophilic substance with the level of functional activity of the adrenal cortex and clarify much of the confusion which has existed in the literature on this subject.[23] During the period when the adrenal cortex is activated to increased activity by A.C.T.H. or stress, the sudanophilic substance of the gland is depleted. When the gland comes to rest after a period of activity this substance reaccumulates. These changes parallel the alterations in the cholesterol content of the gland (*vide infra*).

The high concentration and labile nature of cholesterol in the adrenal has been recognized for some time. The significance of this can now be more clearly understood. Physiological conditions which might be expected to create an increased demand for cortical hormones decrease the concentration of cholesterol in the adrenals.[61] Administration of a single dose of A.C.T.H. results in a prompt fall in the cholesterol content of the adrenal (FIGURE 1). Within three hours after administration of A.C.T.H., the cholesterol content

---

* The experimental work presented in this first part was carried out in collaboration with Dr. C. N. H. Long, Dr. Abraham White, Edith G. Fry, Helen Lewis, and Dr. T. Y. Liang, of the Department of Physiological Chemistry, Yale University School of Medicine.

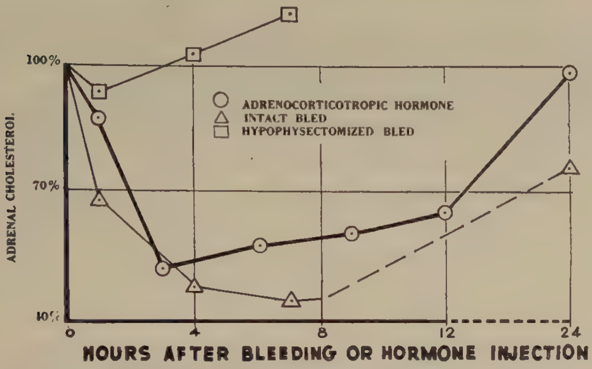


FIGURE 1. Changes in adrenal cholesterol following a single injection of A.C.T.H. (4 mg. per 100 gm. of body weight intraperitoneally) into intact rats and following an acute non-fatal hemorrhage of one hour's duration in intact and hypophysectomized rats. The slight changes which occurred in the cholesterol concentration of the adrenals of hypophysectomized rats are not significant.

of the gland has dropped to 50 per cent of its normal value. After twelve hours, the cholesterol begins to accumulate, and by twenty-four hours has returned to its original concentration. There is a striking similarity between these changes just described and those which follow subjection of the intact rat to a non-fatal hemorrhage. That it is the pituitary which mediates these dynamic changes in adrenal cholesterol is shown by the fact that they do not occur in the hypophysectomized animal exposed to this same stress.

It is ester cholesterol which is subject to the changes just described. Free cholesterol remains unchanged.[39, 53, 65] Other lipid constituents of the adrenal, such as neutral fat[53] and phospholipid,[27, 39, 53] remain unaltered at a time when the cholesterol content is markedly reduced.

The concentration of adrenal ascorbic acid, as well as that of cholesterol, is regulated by the pituitary. Administration of a single dose of A.C.T.H. results in a very prompt depletion of ascorbic acid from the adrenal of the rat (FIGURE 2). Within twelve hours, the original concentration is regained.

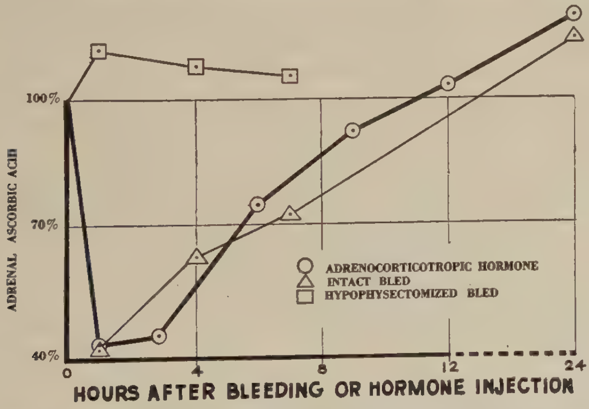


FIGURE 2. Changes in adrenal ascorbic acid following a single injection of A.C.T.H. (4 mg. per 100 gm. of body weight intraperitoneally) into intact rats and following an acute non-fatal hemorrhage of one hour's duration in intact and hypophysectomized rats. The slight increases in the ascorbic acid content of the adrenals of the hypophysectomized rats at 1, 4, and 7 hours are not significant.

Amounts slightly greater than normal are found after twenty-four hours.[*] Following subjection of the intact rat to a severe but non-fatal hemorrhage, the absolute amount of the change in adrenal ascorbic acid as well as the time relationship of the change are strikingly similar to those alterations which follow administration of A.C.T.H. On the other hand, ablation of the pituitary leaves the ascorbic acid content of the adrenal inert and non-responsive when the animal is subjected to this same stress. However, injection of an exogenous source of A.C.T.H. will deplete the ascorbic acid of the adrenal of the hypophysectomized animal.[†]

Recently, both Levin[34] and Ludewig and Chanutin[39] have utilized changes in the cholesterol content of the adrenal to evaluate the response of this organ to several varieties of stress. It has been demonstrated that epinephrine,[16, 36] benzene,[54] estrogens,[43] ether,[6, 33] chloroform,[33] insulin,[48] diphtheria toxin,[19, 71] tetanus toxin,[18] anoxia,[58] infectious diseases,[42] atropine, nicotine, histamine, cold, heat, killed typhoid organisms, hemorrhage, dibenamine, and intraperitoneal administration of glucose[65] all deplete the ascorbic acid content of the adrenal. In the case of histamine, epinephrine, and cold, it has been possible to show that a relationship exists between the intensity of the noxious stimulant and the absolute drop in the concentration of ascorbic acid in the adrenal. The measurement of alterations in adrenal ascorbic acid has proved to be a particularly useful tool for the evaluation of adrenal cortical activity, since there is available a simple and accurate method for the determination of vitamin C.[57] [‡]

An attempt has been made to bring some order out of the large number of varied and sometimes confusing reports on adrenal changes following exposure of animals to a multitude of noxious stimuli and environmental changes. The dynamic nature of the changes observed in the adrenal makes it essential that the following factors be taken into account in an interpretation of the data: (a) the intensity of the stimulation, (b) the duration of the stimulation, and (c) the time during or after stimulation at which the adrenals are analyzed. The responses of the adrenal cortex fall into six general types. The classification is intended as a guide to interpret functional activity of the adrenal cortex on the basis of changes in size, sudanophilic substance, cholesterol, and ascorbic acid of the gland. It is recognized that there are various grades of intermediate responses between these six types.

Type 1. A sudden temporary period of stress (FIGURE 3). In this situation, there is a sudden temporary increased demand for cortical hormones resulting in a temporary increase in pituitary adrenocorticotrophic activity. The sudanophilic substance, the cholesterol, and the ascorbic acid undergo rapid

---

[*] The same dynamic changes in adrenal cholesterol and ascrobic acid have been shown to occur in the guinea pig.[63] As might be expected, however, reaccumulation of ascorbic acid is much slower in the guinea pig than in the rat. Rate of reaccumulation in the guinea pig is influenced by the exogenous supply of this vitamin.

[†] At the present time, vitamin C cannot be assigned a specific role in the processes concerned with the secretion or elaboration of adrenal cortical hormones. Depletion of ascorbic acid may be simply an expression of an overall acceleration of metabolic processes accompanying secretory activity in the gland.

[‡] In small animals such as the rat, analysis of the whole adrenal is made. Separate analyses of adrenal medulla and adrenal cortex have demonstrated that the changes in cholesterol and ascorbic acid do occur in the cortex.[63] The authors have found the alteration in adrenal ascrobic acid to be the same measured by the method of Bessey,[4] which determines reduced ascorbic acid, as that measured by the method of Roe and Keuther,[57] which determines the total (reduced plus dehydro) ascorbic acid.

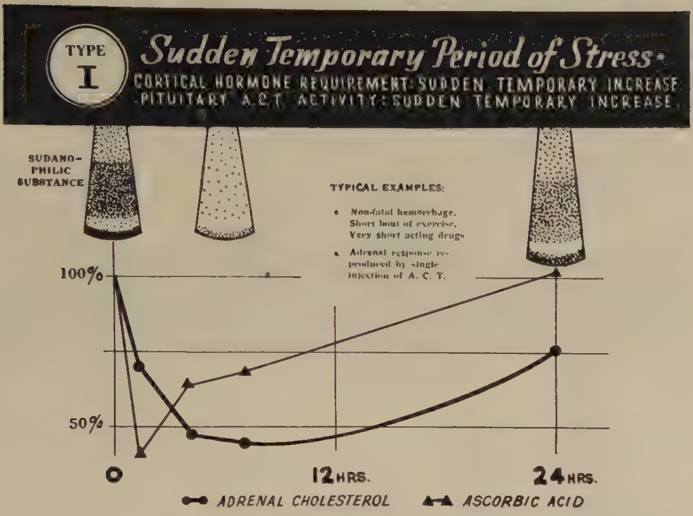


FIGURE 3. Type 1 adrenal response. The actual size of the adrenals is proportional to the total distance the diagrammatic section of the adrenal cortex extends below the upper bar. The changes in sudanophilic substance are taken from the observations of Engel, Winton, and Long.[15] The changes in the cholesterol, ascorbic acid, and size of the gland are those which have been observed to occur after a non-fatal hemorrhage of one hour's duration.[62]

depletion followed by a return of these substances to normal concentration as the animal recovers. A small increase in the size of the adrenal occurs. Typical examples of this type of adrenal response are produced by an acute non-fatal hemorrhage,[62] a short acting bout of muscular exercise,[1, 14, 27] and a single dose of very short acting drugs, such as epinephrine[36] or histamine. The changes in the adrenal characterized as Type 1 can be reproduced by the injection of a single dose of A.C.T.H.

It is evident that a dynamic process involving depletion and mobilization of cholesterol and ascorbic acid is taking place in the adrenals, and that the time of examination of this process is a variable which can quite adequately explain some of the apparent discrepancies which have been reported in the literature.

Type 2. A very slow gradual change in the internal or external environment (FIGURE 4). Here, there is a very slow gradual increase in the demand for cortical hormone which results in an equally gradual increase in pituitary adrenocorticotrophic activity. The concentration of sudanophilic substance, cholesterol, and ascorbic acid of the adrenal remain essentially unchanged. A gradual increase in size of the gland occurs, and it is apparent that new secretory units can be constructed at a rate sufficient to meet the increasing demands. Typical examples of this type are produced by fasting,[40, 44, 47, 49, 50, 68, 72, 78] seasonal changes in temperature, and pregnancy.[2, 7, 55]

Type 3. An intense continuous stress ending in death (FIGURE 5). There is a very great and continuous demand for cortical hormone, resulting in a maximum rate of elaboration of A.C.T.H. from the pituitary. The concen-

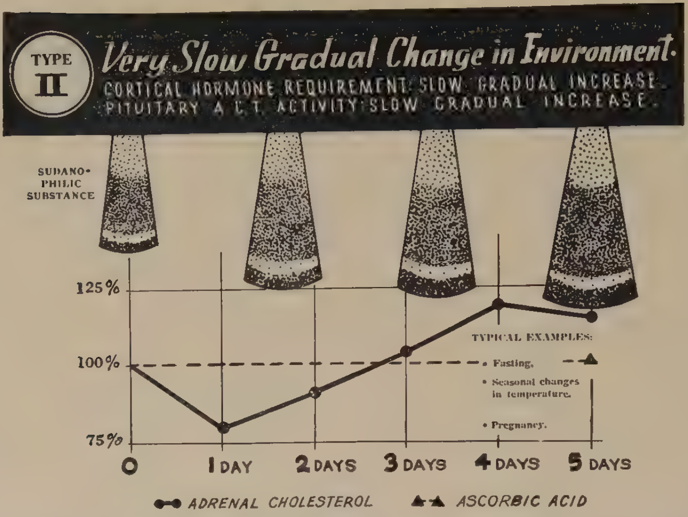


Figure 4. Type 2 adrenal response. The actual size of the adrenals is proportional to the total distance the diagrammatic section of the adrenal cortex extends below the upper bar. The data on sudanophilic substance are taken from the observations of Whitehead[78] on fasting animals. The data on size and cholesterol concentration of the gland represent the actual changes found by Ludewig and Chanutin[39] to occur in fasted rats. The changes which did occur in the cholesterol content of the adrenal are not considered significant. In the case of adrenal ascorbic acid, no data are available for periods of fasting less than five days. However, a fast of six days,[72] seven days,[47] or even 10 days[68] produces no significant change in adrenal ascorbic acid.

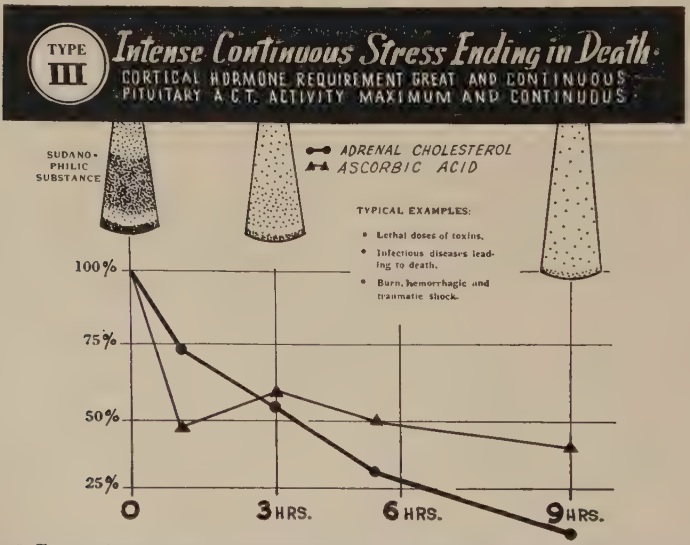


Figure 5. Type 3 adrenal response. The actual size of the adrenals is proportional to the total distance the diagrammatic section of the adrenal cortex extends below the upper bar. The data on sudanophilic substance are taken from the observations of Engel, Winton, and Long[15] on hemorrhagic shock in the rat. The changes in size, cholesterol, and ascorbic acid concentration of the gland are those which occur in rats subjected to hemorrhagic shock.[62]

trations of sudanophilic substance, cholesterol, and ascorbic acid fall rapidly and remain at a very low level until the time of death. The gland is laying down secretory units at a maximum rate, as evidenced by its marked hypertrophy. The amount of this hypertrophy is proportional to the time which elapses between the onset of the stress and death. Typical examples of this type of adrenal response are produced by lethal doses of toxins,[8, 13, 45] infectious diseases leading to a fatal outcome,[3, 5, 11, 13, 17, 20, 26, 30, 31, 32, 38, 42, 60, 67, 75, 76, 79] burn,[9, 21, 52, 73] and hemorrhagic[13, 15, 62] and traumatic[12, 46] shock.

Type 4. Recovery from a period of severe stress or adaptation to stress (FIGURE 6). In this case, the demand for cortical hormone is reduced following a period of stress, and the pituitary returns to a low state of functional activity. During the period of stress, the adrenal becomes enlarged and depleted of sudanophilic substance, cholesterol, and ascorbic acid. Upon removal of the stress, these metabolic constituents accumulate in a manufacturing plant which is over-expanded for the needs of the organism under optimal conditions of the environment. The changes in adrenal size and cholesterol concentration depicted in FIGURE 6 are taken from the data of Ludewig and Chanutin.[39] These investigators determined the concentration of cholesterol in the adrenal each day for several days following the administration of β-ethyl vesicants. The initial depletion of the cholesterol content of the gland represents the toxic phase of the response. The return to normal and subsequent increase of concentration above normal represent the recovery phase.

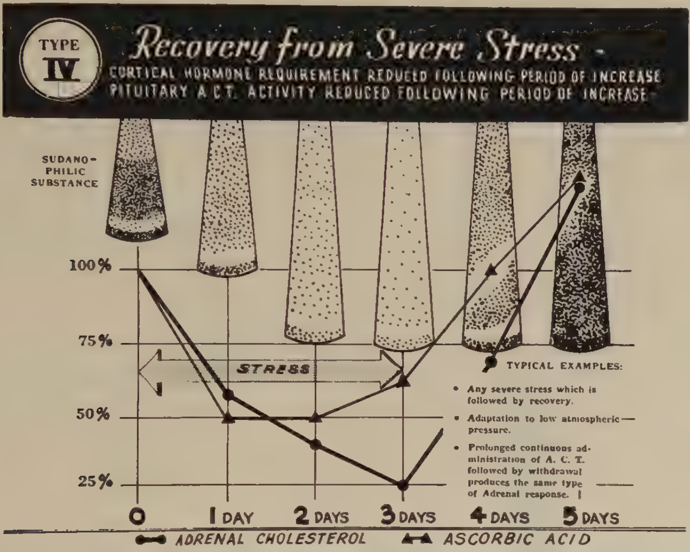


FIGURE 6. Type 4 adrenal response. The actual size of the adrenals is proportional to the total distance the diagrammatic section of the adrenal cortex extends below the upper bar. The changes in size and cholesterol concentration in the gland are taken from the data of Ludewig and Chanutin.[39] The changes in sudanophilic substance[61] and ascorbic acid concentration[63] are changes which take place when an animal is administered A.C.T.H. for a period of three days and then allowed to recover.

This type of adrenal response also occurs when an animal adapts to a continuously applied noxious stimulus. Initially, there is depletion of adrenal constituents, but, as specific adaptations are made, the requirement for cortical hormone is reduced and the gland returns to a lower state of functional activity. For example, Darrow and Sarason[10] demonstrated lipoid depletion of the adrenal cortex during the first five days of exposure of rats to anoxia, followed by restoration of lipoid material on the seventh and eighth days of exposure.

These changes can be reproduced by the continuous administration of A. C. T. H. for a period of a few days, followed by cessation of administration of the hormone.

It is of interest to note that the phase of accumulation of cholesterol and ascorbic acid in a hypertrophied gland corresponds to the period of increased crossed resistance which follows stress.[37]

Type 5. Dysfunction of the pituitary attended by hypersecretion of A.C.T.H. In this case, the organism has no need for increased amounts of cortical hormone, but, due to some malfunction of the pituitary, the adrenal cortex is stimulated to increased secretory activity. The adrenal is maximally increased in size and continuously depleted of its sudanophilic substance, cholesterol, and ascorbic acid. The clinical example of this is Cushing's disease. The picture can be reproduced in the laboratory by chronic administration of large doses of A. C. T. H.

Type 6. Dysfunction of the pituitary attended by hyposecretion of A. C. T. H. In this case, the adrenal cortex atrophies as a result of pituitary hypofunction. The sudanophilic material, the cholesterol, and ascorbic acid of the adrenal become inert and non-responsive to changes in the internal and external environment. A clinical example of this type is found in panhypopituitarism. In the laboratory, it is reproduced by hypophysectomy.

Summary. Data have been accumulated on the effect of A.C.T.H. on the morphology and chemistry of the adrenal gland. These studies have made it possible to correlate levels of functional activity of the adrenal cortex with histological and chemical changes which have been described as occurring in this gland in a multitude of varied stresses and environmental conditions. A classification of the various adrenal responses into six types has been made.

Regulation of Pituitary Adrenocorticotrophic Activity[*]

Whereas the nature of the regulatory control of the pituitary over the adrenal cortex has been definitely established, little is known in regard to the humoral or nervous changes produced by stress which result in rapid elaboration of A.C.T.H. from the hypophysis. Contributions to our knowledge of this problem have been few. Ingle[22] was able to demonstrate an

---

[*] The data presented in this second part were supported by grants from the Utah Copper Company Research Fund, the Rockefeller Fluid Research Fund of the University of Utah, the University of Utah Research Fund, and by a grant from the Committee for Research on Endocrinology, National Research Council.

Grateful acknowledgement is made to Dr. Louis S. Goodman for his able advice and criticism during the course of this investigation.

The authors are indebted to Dr. Wilfred N. Sisk, Dr. Marvin H. Kuizenga, and Dr. Dwight J. Ingle of the Upjohn Company, who made available aqueous adrenal cortical extract, corticosterone, and 17-hydroxy-11-dehydrocorticosterone, to Dr Ernst Oppenheimer of the Ciba Pharmaceutical Products, Inc. for generous gifts of desoxycorticosterone, desoxycorticosterone acetate, and progesterone, and to Dr. C. N. H. Long for a sample of 17-hydroxycorticosterone increase in adrenal size following a 12-hour period of muscular exercise in the rat. Hypertrophy did not occur in those animals treated with adrenal cortical extract during the period of exercise. The experiment may be interpreted to mean that administration of cortical extract abolished the normal stimulus to pituitary activity, namely: a decrease in concentration of cortical hormone(s) in the body fluids. According to Uotila,[74] the adrenal cortical hypertrophy which occurs in rats exposed to cold is not influenced by transection of the infundibulum. The following approach to this problem has been made possible through the availability of a new technique for measuring pituitary adrenocorticotrophic activity.

Changes in the concentration of adrenal ascorbic acid reflect changes in pituitary adrenocorticotrophic activity. It has been demonstrated that the concentration of ascorbic acid in the adrenal is under the specific regulatory control of the A.C.T.H. of the anterior pituitary. Part of the evidence upon which this conclusion is based is presented in FIGURE 7. Administration of a purified preparation of hog A.C.T.H., chemically homogeneous and free of other known pituitary activities, depleted the ascorbic acid content of the adrenals of both the intact and hypophysectomized rat to less than 50 per cent of the control level within a period of one hour following injection. However, whereas application of stress produced the same marked and rapid depletion in the case of the intact rat, it had no influence upon the level of the vitamin in the adrenals of the hypophysectomized rat. The evidence leaves little doubt that the level of ascorbic acid in the adrenal is a reflection of the rate of elaboration of A.C.T.H. from the pituitary.

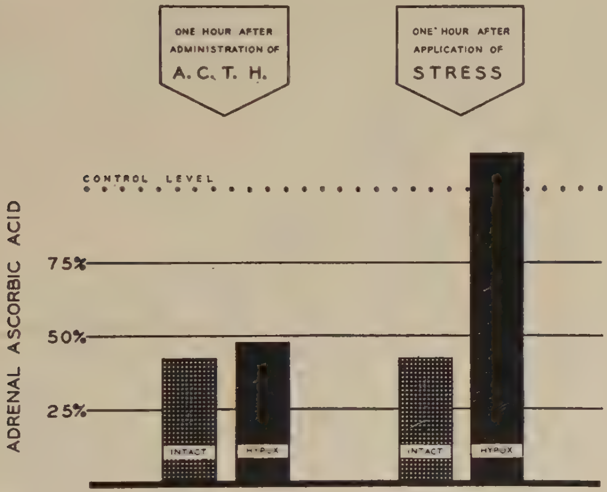


FIGURE 7. Effect of A.C.T.H. and stress (non-fatal hemorrhage of one hour's duration) upon the concentration of ascorbic acid in the adrenals of intact and hypophysectomized rats. The level of the dotted line represents the concentration of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dotted line is proportional to the amount of A.C.T.H. elaborated by the pituitary or to the amount of this trophic hormone injected intraperitoneally. Hypophysectomized is abbreviated to hypox.

The alteration in the concentration of ascorbic acid of the adrenal offers certain advantages as a measure of pituitary adrenocorticotrophic activity. Rapidly fluctuating rates of elaboration of A.C.T.H. can be detected. As can be seen from FIGURE 7, marked depletion of adrenal ascorbic acid took place within an hour following subjection of the intact rat to bleeding. Furthermore, the quantitative nature of the method has made it possible to demonstrate that *the rate of elaboration of A.C.T.H. from the pituitary is proportional to the intensity of stress to which the animal is subjected.* Reducing the temperature of exposure successively from 7 ± 1°C. to 4 ± 1°C., to − 2 ± 1°C., and to −8 ± 1°C. brought about correspondingly greater reductions in adrenal ascorbic acid (FIGURE 8). Likewise, when the dose of injected histamine was increased successively from 0.25 mg. to 0.5 mg., to 1.0 mg., and to 10.0 mg. per 100 gm. body weight, correspondingly greater reductions in the ascorbic acid content of the adrenals took place (FIGURE 9). Superimposed upon the bar charts (FIGURES 8 and 9) is a graph illustrating the direct relationship between the dose of hog A.C.T.H. administered and the reduction in adrenal ascorbic acid. The amount of hormone elaborated by the rat pituitary following exposure to stress can be estimated in terms of activity equivalents of hog A.C.T.H.

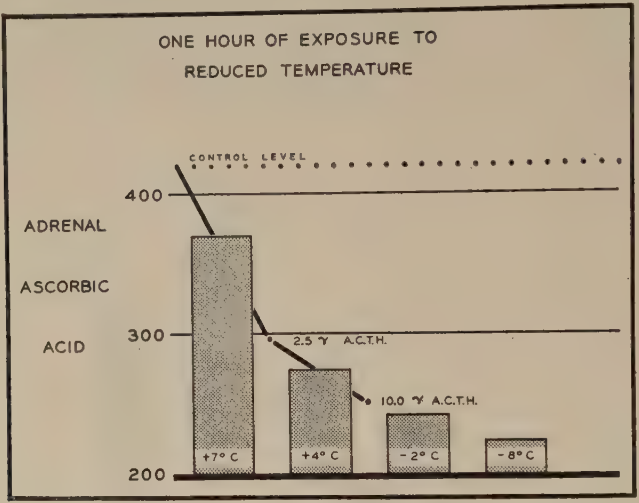


FIGURE 8. The effect of exposure of rats to varying temperatures upon the concentration of ascorbic acid in the adrenals. The level of the dotted line represents the concentration (mg. per 100 gm. of fresh tissue) of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dotted line is proportional to the amount of A.C.T.H. elaborated by the pituitary. Superimposed upon the bar chart is a graph showing the relation between dose of A.C.T.H. (hog) and adrenal ascorbic acid.

Pretreatment with adrenal cortical hormone prevents the depletion of adrenal ascorbic acid which otherwise follows exposure to cold. A rapid elaboration of A.C.T.H. from the pituitary follows the exposure of the rat to cold. As can be seen from FIGURE 10, exposure of the rat to 3°C. for one hour resulted in a marked depletion of adrenal ascorbic acid. Administration of cortical hormone one hour before the beginning of exposure prevented this depletion.

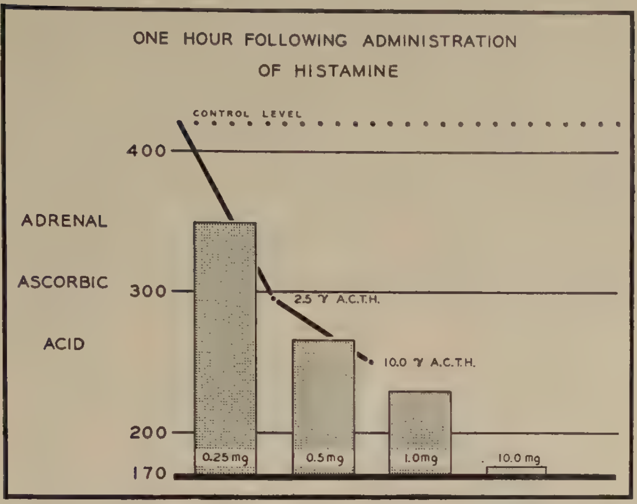


Figure 9. The effect of administration of various doses of histamine acid phosphate upon the concentration of ascorbic acid in the adrenals. The level of the dotted line represents the concentrations of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dotted line is proportional to the amount of A.C.T.H. elaborated by the pituitary. Superimposed upon the bar chart is a graph showing the relation between dose of A.C.T.H. (hog) and adrenal ascorbic acid.

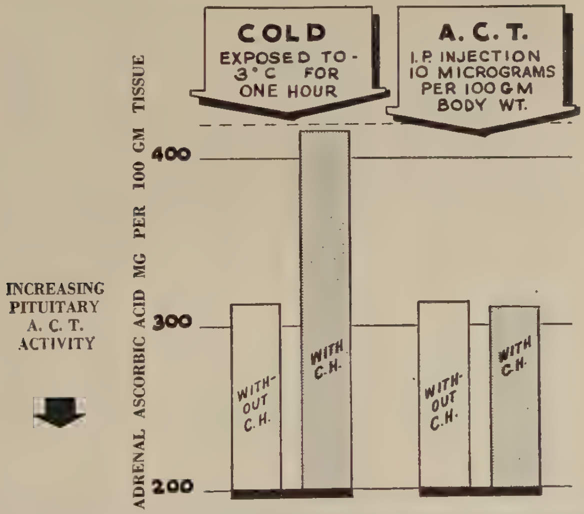


Figure 10. Mode of action of cortical hormone in preventing reduction of adrenal ascorbic acid upon exposure of the rat to stress. The level of the dashed line represents the concentration of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentration of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dashed line is proportional to the amount of A.C.T.H. elaborated by the pituitary or to the amount of this trophic hormone injected intraperitoneally.

A dose of A.C.T.H. was determined which produced the same drop in adrenal ascorbic acid as exposure to 3°C. for one hour. Whereas pretreatment with adrenal cortical hormone prevented the reduction in adrenal ascorbic acid which otherwise follows exposure to cold, it had no effect whatsoever upon the adrenal stimulating effect of the exogenous source of A.C.T.H. (FIGURE 10). Hence, the locus of the inhibitory action of adrenal cortical hormone is not the adrenal cortex itself. By elimination, it would appear that adrenal cortical hormone acts to suppress elaboration of A.C.T.H. from the anterior pituitary.

The phenomenon of inhibition of pituitary adrenocorticotrophic activity by cortical hormones is of a general nature. Not only exposure to cold, but also application of heat, injection of histamine, epinephrine, and killed typhoid organisms brought about a reduction in the concentration of ascorbic acid in the adrenal of the rat (FIGURE 11). This reduction in the vitamin C content of the adrenals indicates that each one of these stresses, acting for a period of one hour, stimulated the anterior pituitary to elaborate A.C.T. H. On the other hand, if the animals were administered cortical hormone immediately prior to the application of the stress, no reduction in the concentration of adrenal ascorbic acid took place.

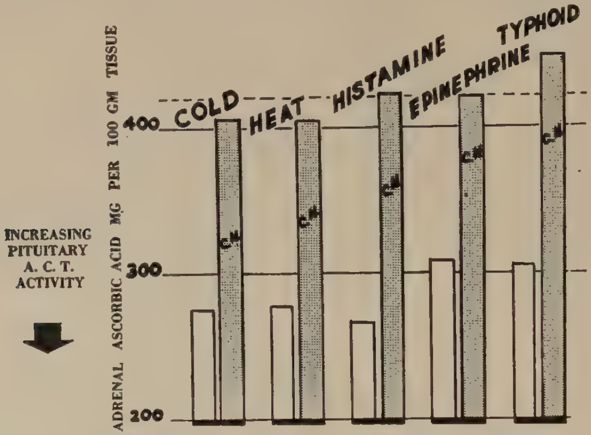


FIGURE 11. General nature of the phenomenon of pituitary inhibition by cortical hormones. The level of the dashed line represents the concentration of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dashed line is proportional to the amount of A.C.T.H. elaborated by the pituitary. The unshaded columns represent the changes which took place without the cortical hormone treatment and the shaded columns the changes with cortical hormone treatment. The concentrations of ascorbic acid in the adrenals of rats treated with cortical hormones as represented by the shaded columns are not significantly different, in any case, from the concentration of ascorbic acid in the adrenals of untreated rats.

The administration of cortical hormone suppressed the enhanced pituitary adrenocorticotrophic activity which otherwise accompanies environmental change (heat, cold), drug administration (histamine, epinephrine), and bacterial intoxication (injection of a suspension of killed typhoid organisms). Hence, if the animal is furnished with an adequate exogenous source of adrenal cortical hormone during exposure to any one of a number of stresses, the normal stimulus to adrenal cortical activity is abolished. It would appear reasonable to suppose that the great variety of non-specific stresses increases pituitary adrenocorticotrophic activity by a common mechanism, namely, by increasing the requirement of the peripheral tissue cells for cortical hormone(s). The anterior pituitary responds to the decrease in concentration of cortical hormone in the body fluids by increasing the rate of elaboration of A.C.T.H. It is recognized that in addition to the pituitary itself, some other intermediary structure may be involved. The hypothalamus, for example, rather than the pituitary, might interpret the blood levels of cortical hormone and make the appropriate adjustment in pituitary adrenocorticotrophic activity *via* nervous pathways. As mentioned previously, however, Uotila[74] found that section of the infundibulum did not interfere with the hypertrophy of the adrenal cortex which accompanies chronic exposure to cold.

Certain quantitative relationships hold for this regulatory mechanism. It has been possible to demonstrate (FIGURE 12) that *the degree of inhibition of pituitary adrenocorticotrophic activity is proportional to the amount of administered cortical hormone.* As can be seen from FIGURE 12, 0.2 ml. of cortical extract per 100 gm. of body weight prevented the reduction in adrenal ascorbic acid which otherwise followed exposure of rats to 3°C. On the other hand, 0.01 ml. had very slight, if any, and 0.1 ml. had only partial blocking effect.

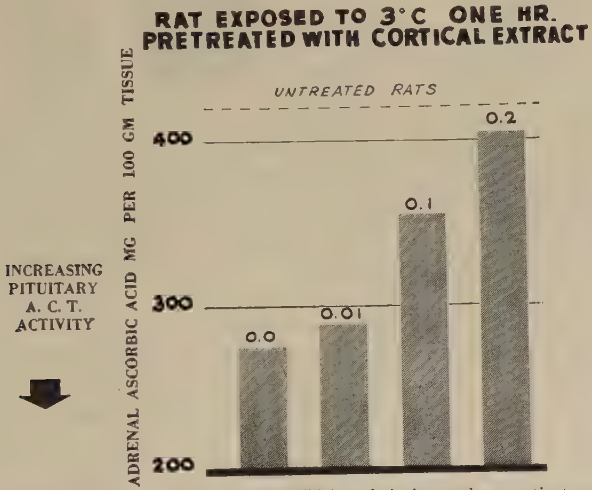


FIGURE 12. The relation between the degree of inhibition of pituitary adrenocorticotrophic activity and the dose of cortical hormone administered. The level of the dashed line represents the concentration of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of a column extends below the dashed line is proportional to the amount of A.C.T.H. elaborated by the pituitary. The figures atop the columns represent the milliliters of aqueous cortical extract (Upjohn) per 100 grams of body weight injected subcutaneously one hour before exposure to cold.

It has also been possible to demonstrate (FIGURE 13) that the *greater the degree of stress to which the animal is subjected the greater is the amount of cortical hormone required to inhibit pituitary adrenocorticotrophic activity.* Whereas 20 micrograms of 17-hydroxycorticosterone per 100 gm. of body weight completely inhibited the increase in adrenal cortical activity which otherwise follows injection of 0.25 mg. of histamine, this dose of steroid produced only partial inhibition in rats treated with 0.5 mg. of histamine.

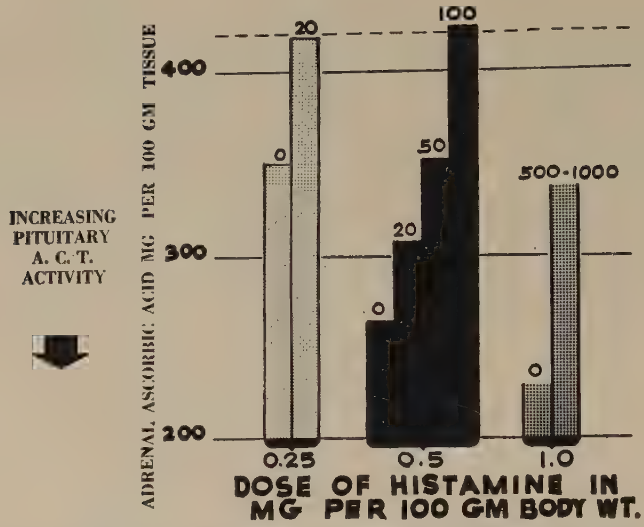


FIGURE 13. Relations between degree of stress and the dose of cortical hormone required to partially or completely inhibit pituitary adrenocorticotrophic activity. The level of the dashed line represents the concentration of ascorbic acid in the adrenals of untreated rats. The height of the columns represents the concentrations of ascorbic acid in the adrenals of treated rats. The distance the top of the column extends below the dashed line is proportional to the amount of A.C.T.H. elaborated by the pituitary. The figures atop the columns represent the amount of 17-hydroxycorticosterone in micrograms per 100 grams of body weight injected subcutaneously one hour before histamine administration.

The greater pituitary adrenocorticotrophic activity produced by this larger dose of histamine could be blocked by administration of 100 micrograms of 17-hydroxycorticosterone. In animals treated with 1.0 mg. of histamine, a dose of 1.0 mg. of 17-hydroxycorticosterone resulted in only partial suppression of pituitary activity. No more than a guess may be made at the present time as to the reason why even large doses of cortical hormone were unable to bring about complete inhibition of pituitary activity following the administration of 1.0 mg. of histamine per 100 gm. of body weight. At least two possibilities exist: (1) complete inhibition requires still larger doses than those used; (2) some factor, other than the rate of utilization of cortical hormone, comes into play in severe stress to excite the pituitary.

From these data, we may interpret the interrelationships among the pituitary, the adrenal cortex, and the tissue cells as diagrammatically presented in FIGURE 14. Under optimal conditions of the environment, the pituitary-adrenal system is in a relatively quiescent state. The peripheral tissue cells, under these conditions, require relatively small amounts of cortical hormone(s), and the concentration of the latter in the circulating blood is maintained at a level which holds the anterior pituitary in check. This means that, under optimal conditions of the environment, minimum amounts of A.C.T.H. are elaborated by the anterior pituitary and minimum amounts of cortical hormone(s) are secreted by the adrenal cortex.

This is to be contrasted with the activity of the pituitary-adrenal system which occurs under conditions of stress. The requirement for cortical hor-

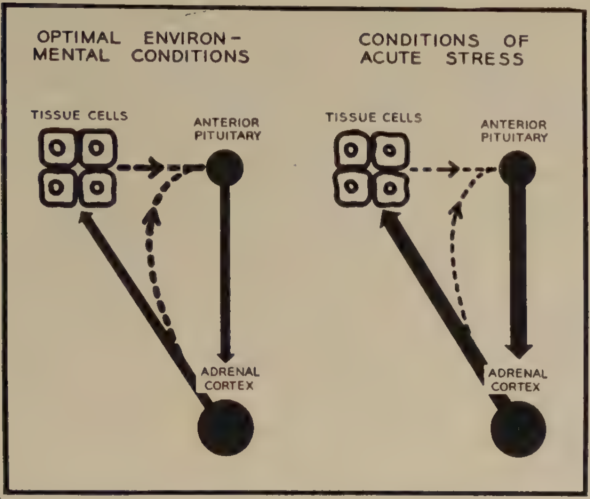


FIGURE 14. The pituitary-adrenal system under optimal environmental conditions and under conditions of stress. The width of the lines is proportional to the concentration of hormone in the body fluids. The two dashed lines, one off to the side of the tissue cells, and the other going directly from the tissue cells to the pituitary, indicate that the cortical hormones may have a direct inhibitory effect on the pituitary or that they may have an indirect effect through some product of their metabolic action on the tissue cells.

mone(s) by the tissue cells is accelerated and hence, the quantity in the circulating blood is reduced. The check on the anterior pituitary is now removed and A.C.T.H. is elaborated in relatively large quantities. Increased secretory activity of the adrenal cortex occurs, meeting the needs of the peripheral tissue cells for the cortical steroids. This increased activity of the pituitary-adrenal system continues until the stress is removed or until adaptation occurs, at which time, the requirement of the peripheral tissue cells for cortical hormone(s) is diminished.

The quantitative relationships which hold for this regulatory mechanism may be adequately explained if it is assumed that the rate of elaboration of A.C.T.H. from the pituitary can fluctuate by gradual change and according to the requirements of the tissue cells for cortical steroids. Just as the pituitary-thyroid system maintains the tissues in a state of well-being with regard to thyroid hormone, so the pituitary-adrenal system maintains the tissues in a state of well-being with regard to adrenal cortical hormone(s). The term, euthyroidism, has been used to denote a state of well-being of the tissues with regard to thyroid hormone.[59] "Eucorticism" would be a comparable term to apply to the adrenal cortical hormone(s).

Relative pituitary inhibitory potency of crystalline steroids. An estimate of the pituitary inhibitory potency of five crystalline steroids has been made. The approximate potency rating has been assigned on the basis of the comparative ability of these substances to block the pituitary adrenocorticotrophic activity which accompanies exposure to a uniform degree of cold or which follows injection of a standard dose of histamine. It is to be noted that the experiments were of short duration. A period of one hour elapsed between injection of the steroid and subjection of the animal to stress.

Hence, the estimated potencies of these steroids are undoubtedly influenced by their relative solubilities in the vehicle as compared to the tissue fluids surrounding the subcutaneous site of injection.

Corticosterone has been assigned a pituitary inhibitory potency rating of one (TABLE 1). Seventeen-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone are of equal potency and are about four times as active as corticosterone. Desoxycorticosterone, despite limitations in regard to solubility, is approximately one-half as potent as corticosterone, while progesterone has only $\frac{1}{50}$ the activity of corticosterone. It is apparent that both types of cortical steroids, the C_{11} oxygenated and the C_{11} desoxy type, possess pituitary inhibitory potency. In TABLE 1, the relative potencies of the five steroids in regard to sodium retention and glycogenesis are also presented. The most potent compounds have been given a rating of 100. There is no parallelism between the effect of the steroids on electrolyte metabolism, carbohydrate metabolism, and their ability to inhibit pituitary adrenocorticotrophic activity. Desoxycorticosterone, 17-hydroxycorticosterone, and 17-hydroxy-11-dehydrocorticosterone all have pituitary inhibitory potency, but, whereas desoxycorticosterone produces sodium retention and has no glycogenetic activity, 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone produce sodiumphoresis and are most potent in glycogenetic activity. It is of interest, in this connection, that Ingle and Kendall[25] came to the conclusion that the level of sodium or potassium in the body fluids does not directly influence pituitary adrenocorticotrophic activity.

TABLE 1

RELATION BETWEEN METABOLIC ACTIVITY AND PITUITARY INHIBITORY POTENCY OF FIVE CRYSTALLINE STEROIDS (SEE TEXT FOR EXPLANATION)

Steroid Hormone	a	b	c
	Sodium Retention	Glycogenesis	Pituitary Inhibition
17-Hydroxy-corticosterone	0	100	4
17-Hydroxy-11-Dehydro-corticosterone	0	90	4
Corticosterone	50	67	1
11-Desoxy-corticosterone	100	0	$\frac{1}{2}$
Progesterone	5†	0*	$\frac{1}{50}$

a. Ingle.[24]
b. Olson *et al.*[51]
* Lacks diabetogenic and anti-insulin activities.[56]
† Thorn and Engel.[70]

If the "direct action" hypothesis is correct, then it necessarily follows that administration of desoxycorticosterone in suppressing pituitary adrenocorticotrophic activity should result in a deficiency of endogenously secreted C_{11} oxygenated steroids. There is evidence to suggest that this is indeed true. Clinical reports indicate that DOCA increases the tolerance to glucose of diabetic patients.[28, 29, 41, 77] Selye *et al.*[66] have found that chronic treatment with DOCA produces adrenal cortical atrophy and impairs the ability of the rat to mobilize glucose when subjected to a variety of non-specific types of stress.

Further investigation of the relation between metabolic activity, chemical structure, and pituitary inhibitory potency is being conducted at the present time. The data which have been obtained so far suggest, but do not offer conclusive proof, that cortical steroids have a direct action on the pituitary rather than an indirect one through some product of their metabolic activity or through their deficiency.

Summary. The administration of cortical hormone suppressed the enhanced pituitary adrenocorticotrophic activity which otherwise accompanies environmental change (heat, cold), drug administration (histamine, epinephrine), and bacterial intoxication (injection of a suspension of killed typhoid organisms). The data may be interpreted to mean that the great variety of non-specific stresses increases pituitary adrenocorticotrophic activity by a common mechanism, namely, by increasing the requirement of the peripheral tissue cells for cortical hormone(s). The hypophysis responds to the decrease in concentration of cortical hormone in the body fluids by increasing the rate of elaboration of trophic hormone.

Certain quantitative relationships hold for this regulatory mechanism. First, the degree of inhibition of pituitary adrenocorticotrophic activity is proportional to the amount of administered cortical hormone. Second, the greater the degree of stress to which the animal is subjected, the greater is the amount of cortical hormone required to inhibit pituitary adrenocorticotrophic activity. These quantitative data may be adequately explained if it is assumed that the rate of elaboration of A.C.T.H. from the pituitary can fluctuate by gradual change and according to the requirements of the tissue cells for cortical steroids. It is suggested that this regulatory mechanism maintains the tissue cells in a state of "eucorticism" during environmental change and exposure to moderate degrees of stress which the organism encounters in its everyday existence.

Since pituitary inhibitory potency resides in both the C_{11} oxygenated and the C_{11} desoxy type steroids, it is suggested, that the steroids act directly on the anterior pituitary rather than indirectly through products of their metabolic activity or their deficiency.

Bibliography

1. Anderson, D. H. 1935. J. Physiol. **85:** 162.
2. Anderson, D. H., & W. M. Sperry. 1937. J. Physiol. **90:** 296.
3. Baumann, E. J., & O. M. Holly. 1925. J. Biol. Chem. **63:** lxiii.
4. Bessey, O. H. 1938. J. Biol. Chem. **126:** 771.
5. Blumensaat, C. 1929. Arch. path. Anat. (Virchow's) **271:** 639.
6. Bowman, D. E., & E. Muntwyler. 1937. Proc. Soc. Exp. Biol. & Med. **35:** 557.
7. Chamberlain, E. N. 1929. J. Physiol. **68:** 259.
8. Clevers & Goormaghtigh. 1922. Bull. Acad. Roy. Méd. Belg. **2:** 425.
9. Crema, C. 1928. Boll. Soc. ital. biol. sper. **3:** 59.
10. Darrow, D. C., & E. L. Sarason. 1944. J. Clin. Invest. **23:** 11.
11. Deanesly, R. 1931. Am. J. Anat. **47:** 475.
12. Donahue, J. K., & W. M. Parkins. 1935. Proc. Soc. Exp. Biol. & Med. **32:** 1249.
13. Elliott, T. R. 1914–1915. Quart. J. Med. **8:** 47.
14. Elliott, T. R., & I. Tuckett. 1906. J. Physiol. **34:** 332.
15. Engel, F. L., M. G. Winton, & C. N. H. Long. 1943. J. Exp. Med. **77:** 397.

16. Euler, H. v., & E. Klussmann. 1933. Z. physiol. Chem. **217:** 167.
17. Ewart, B. 1934–35. Upsala Lakarforon Forh. **40:** 425.
18. Ghosh, B. N. 1939. J. Indian Chem. Soc. **16:** 657.
19. Ghosh, B. N. 1939. J. Indian Chem. Soc. **16:** 241.
20. Graham, G. S. 1916. J. Med. Res. **34:** 214.
21. Harkins, H. N., & C. N. H. Long. 1945. Am. J. Physiol. **144:** 661.
22. Ingle, D. J. 1938. Am. J. Physiol. **124:** 627.
23. Ingle, D. J. 1942. Endocrinology **31:** 419.
24. Ingle, D. J. 1944. *The Chemistry and Physiology of Hormones.* A.A.A.S. Washington, D. C.
25. Ingle, D. J., & E. C. Kendall. 1938. Am. J. Physiol. **122:** 585.
26. Karoling, L. 1925. Magyar Orvosi Arch. **25:** 495.
27. Knouff, R. A., J. B. Brown, & B. M. Schneider. 1941. Anat. Rec. **79:** 17.
28. Kohler, V., & A. Fleckenstein. 1942. Deut. Arch. Klin. Med. **189:** 530.
29. Kohler, V., & A. Fleckenstein. 1941. Klin. Wochenschr. **20:** 844.
30. Lage, H. zur. 1940. Monatsschr. Kinderheilk. **82:** 91.
31. Laignel-Lavastine, M. 1918. C. R. Soc. Biol. **81:** 324.
32. Landau, M., & J. W. McNee. 1914. Beitr. path. Anat. **58:** 667.
33. Lauber, H. J., H. Dumke, & A. Patzsche. 1937. Z. ges. exp. Med. **102:** 1.
34. Levin, L. 1945. Endocrinology **37:** 34.
35. Li, C. H., H. M. Evans, & M. E. Simpson. 1943. J. Biol. Chem. **149:** 413.
36. Long, C. N. H., & E. G. Fry. 1945. Proc. Soc. Exp. Biol. & Med. **59:** 67.
37. Lowenstein, B. E., & R. L. Zwemer. 1942. Endocrinology. **30:** S1035.
38. Lucke, B., T. Wight, & E. Kime. 1919. Arch. Internal Med. **24:** 154.
39. Ludewig, S., & A. Chanutin. 1946. Endocrinology **38:** 376.
40. MacLachlan, P. L., H. C. Hodge, & R. Whitehead. 1941. J. Biol. Chem. **139:** 185.
41. McCavack, T. H., G. P. Charlton, & S. Klotz. 1941. J. Clin. Endocrinol. **1:** 824.
42. Menten, M. L., & M. P. Smith. 1936. Am. J. Dis. Children **52:** 54.
43. Mlinko, Z. 1938. Z. physiol. Chem. **256:** 42.
44. Mouriquand, G., & A. Leulier. 1927. J. Physiol. Path. Gen. **25:** 308.
45. Mouriquand, G., A. Leulier, & B. Sudallian. 1929. C. R. Soc. Biol. **99:** 1923.
46. Muirhead, E. E., C. T. Ashworth, L. A. Kregel, & J. M. Hill. 1942. Arch. Surg. **45:** 863.
47. Mulinos, M. G., L. Pomerantz, & M. E. Lojkin. 1942. Endocrinology **31:** 276.
48. Ohta, K. 1938. Arb. Med. Fakultat Okayama. **6:** 1.
49. Okuneff, N. 1922. Beitr. Path. Anat. **71:** 99.
50. Oleson, M. C., & W. R. Bloor. 1941. J. Biol. Chem. **141:** 349.
51. Olson, R. E., S. A. Thayer, & L. J. Kopp. 1944. Endocrinology **35:** 464.
52. Pfeiffer, H. 1919. Z. Exp. Med. **10:** 1.
53. Popjak, G. 1944. J. Path. & Bact. **56:** 485.
54. Poumeau-Delille, G. 1941. C. R. S. B. **135:** 1276.
55. Randall, L. O., & M. Graubard. 1940. Am. J. Physiol. **131:** 291.
56. Reichstein, T., & C. W. Shoppe. 1943. Vitamins and Hormones **1:** 345.
57. Roe, J. H., & C. A. Kuether. 1943. J. Biol. Chem. **147:** 399.
58. Sacerdote, P. 1938. Boll. soc. ital. biol. sper. **13:** 847.
59. Salter, W. T. 1944. *The Chemistry and Physiology of Hormones* **104:** Science Press. Lancaster.
60. Sarason, E. L. 1943. Arch. Internal Med. **71:** 702.
61. Sayers, G., M. A. Sayers, E. G. Fry, A. White, & C. N. H. Long. 1944. Yale J. Biol. Med. **16:** 361.
62. Sayers, G. M. A. Sayers, T. Y. Liang, & C. N. H. Long. 1945. Endocrinology **37:** 96.
63. Sayers, G., M. A. Sayers, T. Y. Liang, & C. N. H. Long. 1946. Endocrinology **38:** 1.
64. Sayers, G., A. White, & C. N. H. Long. 1943. J. Biol. Chem. **149:** 425.
65. Sayers, M. A., & G. Sayers. Unpublished data.
66. Selye, H., & C. Dosne. 1942. Endocrinology **30:** 581.
67. Sperry, W. M., & V. A. Stoyanoff. 1935. J. Nutrition **9:** 131.
68. Sure, B., & R. M. Theis. 1939. Endocrinology **24:** 673.
69. Tepperman, J., F. L. Engel, & C. N. H. Long. 1943. Endocrinology **32:** 373.
70. Thorn, G. W., & L. L. Engel. 1938. J. Exp. Med. **68:** 299.
71. Torrance, C. C. 1940. J. Biol. Chem. **132:** 575.
72. Tyslowitz, R. 1943. Endocrinology **32:** 103.

73. URECHIA, G. I., I. MANTA, & BUMBACESCU. 1940. Monatsschr. Psychiat. Neurol. **102:** 143.
74. UOTILA, U. U. 1939. Endocrinology **25:** 605.
75. WACKER, L., & W. HUECK. 1913. Arch. Exp. Path. & Pharmakol. **71:** 373.
76. WALTMAN, O. 1913. Beitr. Path. Anat. **56:** 278.
77. WINNETT, E. B., J. W. CALDWELL, & J. E. KAHLER. 1940. J. Iowa State Med. Soc. **30:** 45.
78. WHITEHEAD, R. 1942. J. Path. & Bact. **54:** 169.
79. WHITEHEAD, R. 1934. Brit. J. Exp. Path. **15:** 279.

THE CHEMISTRY AND PARTIAL SYNTHESIS OF ADRENAL STEROIDS

By EDWARD C. KENDALL*

Division of Biochemistry, Mayo Foundation, Rochester, Minnesota

It is just ten years since Steiger and Reichstein[1] prepared desoxycorticosterone by partial synthesis. It is fortunate that the yield of this steroid and the supply of starting material were sufficient to permit preparation of large amounts of this hormone so that its physiological activity could be determined both in experimental animals and in patients who had Addison's disease. These studies soon indicated that, although there was a marked effect on the metabolism of sodium, potassium, chloride, and water, desoxycorticosterone produced but little effect on the metabolism of fat, carbohydrate, and protein. It did not markedly increase the resistance of animals to stress, toxic compounds, and infections. There was a wide discrepancy between the physiological effect of extracts of the adrenal cortex and that of desoxycorticosterone.

At the same time, it was shown that the crystalline hormones of the adrenal cortex with an atom of oxygen at C_{11}, in experimental animals, did influence the metabolism of fat, carbohydrate, and protein, and also increased the resistance to stress, toxic compounds, and infections. However, the amounts of material available were insufficient to extend these observations to a clinical study of patients who had Addison's disease.

In 1940, since it seemed probable that the hormones of the adrenal cortex would be useful therapeutic agents in the treatment of shock and trauma encountered in military operations, several laboratories throughout the world started investigations with the objective of preparing these hormones by partial synthesis. A survey of available starting material indicated that plant steroids could not readily be used, since oxygen could not be added at C_{11}. However, the bile acids provided a source of steroids which theoretically could be rearranged with an atom of oxygen at C_{11}.

Desoxycholic acid appeared to be a practical starting material, and experience has shown that this conclusion was correct. The conversion of desoxycholic acid to hormones of the adrenal cortex involves four changes, each one of which is composed of from two to twelve steps. These changes are: (1) introduction of oxygen at C_{11}; (2) removal of the side chain at C_{20}; (3) elaboration of the ketol group at C_{20}:C_{21}; and (4) formation of an unsaturated ketone in ring A.

The sequence of the steps which have been used in the conversion of desoxycholic acid to the hormones of the adrenal cortex is as follows: In the preparation of dehydrocorticosterone, Lardon and Reichstein[2] (1) removed the side chain; (2) introduced oxygen at C_{11}; (3) elaborated the ketol group at C_{20},:C_{21}; and (4) formed a double bond ketone in ring A. Von Euw, Lardon, and Reichstein,[3] in the partial synthesis of corticosterone, (1) removed the side chain; (2) introduced the hydroxyl group at C_{11}; (3) elaborated a ketol group at C_{20}:C_{21}; and (4) formed a double bond ketone in ring A. For the partial synthesis of 17-hydroxydehydrocorticosterone, Sarett[4] (1) removed the side chain; (2) introduced oxygen at C_{11}; (3) elaborated the trihydroxy group C_{17}:C_{20}:C_{21}; (4) formed the double bond ketone in ring A; and (5) converted the hydroxyl group at C_{20} to a ketone.

---

* This work has been made possible by the cooperation of the following: B. F. McKenzie, W. F. McGuckin, L. L. Engel, R. B. Turner, V. R. Mattox, and G. A. Fleisher.

The partial synthesis of these three hormones furnished evidence for the structures previously assigned on the basis of their chemical properties, but the yields precluded the use of the original methods for the commercial preparation of these hormones. The only possibilities to make them available for use in clinical medicine are to increase the yield by the method of preparation originally used or to find better methods of preparation. It is my purpose, at this time, to report the progress which has been made in the partial synthesis of the hormones of the adrenal cortex as of today.

Introduction of Oxygen at C_{11}. In the early investigation, the problem of major importance was introduction of oxygen at C_{11}. Reichstein and his associates[5, 6] accomplished this by formation of the double bond C_{11}:C_{12} by pyrolysis of methyl 3-keto-12-benzoxycholanate which was prepared from desoxycholic acid. Reduction of the ketone at C_3 and addition of hypobromous acid followed by oxidation yielded the 11-keto-12-bromo compound. Reductive debromination gave the 11-keto derivative. There is a large amount of labor involved in this method for introduction of oxygen at C_{11}. Since this initial work, two other satisfactory methods have been devised.

Several years ago, Marker and Lawson,[7] and also Longwell and Wintersteiner,[8] investigated the replacement of the atom of bromine in methyl 3-acetoxy-11-bromo-12-ketocholanate. They prepared a dihydroxyketo compound which was designated as a 3(α),11-dihydroxy-12-ketocholanic acid. Gallagher[9] prepared the (α) and (β)-11-monobromo derivatives of methyl 3(α)-acetoxy-12-keto-cholanate in crystalline form and from these prepared the 11(α) and (β)-hydroxy-12-keto compounds. He then showed that if these compounds were boiled in alkaline solution a rearrangement of the ketol occurred with formation of the 11-keto-12-hydroxy compound in a yield of approximately 70 per cent. This investigation clarified the results of the early investigators. The 11-hydroxy compound is obtained under mild conditions when the atom of bromine is replaced with a hydroxyl group; but at a higher temperature, the rearrangement of the ketol readily occurs and the acid previously described as the 11-hydroxy-12-keto compound is, in fact, the 11-keto-12-hydroxy derivative. Subsequently, Borgstrom and Gallagher[10] showed that the hydroxyl group at C_{12} could be replaced through the action of phosphorus tribromide with formation of the 11-keto-12(α)-bromo derivative. Reductive debromination yielded the 11-keto compound.

The other method for the introduction of oxygen at C_{11} was devised in my laboratory.[11] The basis for this method is the replacement of the atom of bromine at C_{11} in an 11,12-dibromo derivative. It had previously been found that, when 3(α)-hydroxy-11,12-dibromocholanic acid was heated with sodium hydroxide, a double bond was introduced at C_9:C_{11}, and the atom of bromine at 12 was replaced with the hydroxyl group.[12, 13] Subsequently, it was found that, if the atom of hydrogen at 9 was replaced with a cyclic ether 3,9, then a double bond 9,11 could not be formed, and treatment of the 11(β),12(α)-dibromo compound resulted in an almost quantitative substitution of bromine at C_{11} with oxygen. The 3,9-cyclic ether was subsequently opened with hydrogen bromide, and methyl 3(α)-acetoxy-11-keto-12(α)-bromocholanate was separated in good yield. The interesting feature of this method for introduction of oxygen at C_{11} is the surprisingly high yields for the intermediate compounds. The details of these reactions have been published and will not be repeated in this paper.[11, 13–17]

Since the series of reactions was first devised, this method has been found satisfactory for the preparation of large amounts of steroids with oxygen at C_{11}.

Removal of the Side Chain. Until recently, the most satisfactory method for removing the side chain at C_{20} was through the stepwise degradation devised by Barbier and Locquin[18] and adapted to the steroids by Wieland and his associates.[19] During work on this phase of the problem in my laboratory, significant improvements were made in the use of this method.[11] The usual yield in the past for the formation of the diphenylcarbinol has been 50 to 60 per cent. Dehydration of the carbinol to yield a diphenylethylene was carried out without significant loss, but the oxidation of the diphenylethylene to the nor acid usually gave yields of the order of 50 to 60 per cent.

At a low temperature, from 0° to 5°, it was found that the methyl 3,9-epoxy-11-keto-12-bromocholanate formed the diphenylcarbinol in a yield of more than 90 per cent. The atom of bromine at C_{12} adjacent to the ketone was simultaneously removed[20] through the action of the Grignard reagent. This formed a convenient method to prepare the debrominated product without the need of an extra step. The yield of the diphenylethylene from the carbinol was practically quantitative, and the oxidation of the diphenylethylene to the nor acid was accomplished in a yield of more than 90 per cent. In other words, from the cholanic acid to the nor acid the yield was raised to approximately 80 per cent. The degradation of the second atom of carbon to give the bisnor acid was carried out in comparable yields, and the conversion of the bisnor ester to the ethylene with double bond C_{20}:C_{22} was accomplished in a yield of approximately 75 per cent.

Within the past three years, Miescher and associates have described a new method to shorten the labor involved for the degradation of the side chain at C_{20}. Ziegler and associates[21] previously had studied bromination of unsaturated compounds with bromosuccinimide. When bromination of the diphenylethylene was attempted by the use of bromosuccinimide, the yields were too low to compete with the degradation through the Barbier-Wieland procedure. Recently, in Miescher's[22] laboratory, it was found that good yields of the 22-monobromo derivative of the diphenylethylene could be obtained if the bromination was carried out in the presence of light. Subsequent dehydrobromination gave the diene Δ.[20, 22; 23, 24] Through this procedure, in two steps which were carried out in a single operation, a double bond was introduced at C_{20}:C_{22} and a single oxidation furnished the ketone at C_{20}. However, with the compounds under consideration, the over-all yield of the 20-ketone through this method was far from quantitative. To the present, the best yield has been in the neighborhood of 38 per cent. Since 34 per cent could be obtained through the stepwise degradation, there is not much advantage in the use of the diene, except that the amount of labor involved is much less.

Elaboration of the Ketol. For elaboration of the ketol group at C_{20}:C_{21}, Reichstein and his associates have used two methods. In the preparation of dehydrocorticosterone[2] the 20-ketone with a methyl group at C_{21} was converted to the etio acid by removal of the 21-methyl group. Through the acid chloride of the etio acid thus obtained, diazomethane afforded the diazoketone, which was, in turn, converted to the 21-acetoxyl, 20-ketone compound. The other method was the direct formation of the 21-acetoxyl group from 3,11-dihydroxy-20-ketopregnane.[3] This was accomplished with lead tetracetate.

After Miescher and associates had devised a method to form the $\Delta^{20,22;23,24}$ derivative, it appeared possible from previous work in my laboratory that further bromination would yield either the C_{21}-bromo or C_{17}-bromo compound. In either case, the product could be used to advantage. Bromination of 3(α)-acetoxy-11-keto-12-bromo-24,24-diphenyl-$\Delta^{20,22;23,24}$-choladiene with bromosuccinimide was therefore tried, and it was found that bromine substituted hydrogen at C_{21}. This 12,21-dibromo compound of the diene separated in pure form in a yield of approximately 62 per cent. This is in contrast to the preparation of the $\Delta^{20,22;23,34}$-diene from 3(α)-acetoxy-11-keto-12-bromo-24,24-diphenyl-$\Delta^{23,24}$-cholene. These two last-mentioned compounds form a mixture which cannot readily be separated. All attempts to prepare a pure sample of the $\Delta^{20,22;23,24}$-diene have been unsuccessful. Neither crystallization from various solvents, nor chromatography, has yielded a pure sample of the diene. However, the introduction of the bromine at C_{21} modified the ease of crystallization and the 12,21-dibromodiene separated readily.

It was also found that the atom of bromine at C_{21} in methyl 3(α)-acetoxy-11-keto-12,21-dibromo-24,24-diphenyl-$\Delta^{20,22;23,24}$-choladiene is replaced very easily either with the acetoxyl or with the methoxyl group. The 21-acetoxy and 21-methoxy compounds have been separated in pure crystalline form. Oxidation with chromic acid gave the 20-ketone with the acetoxyl or methoxyl group at C_{21}. It has also been found that in methanol the 21-bromodiene, with an acetoxyl group at C_3, was converted into the 3(α)-hydroxy-21-methoxy compound. This, in turn, with hydrogen bromide, was converted in excellent yield to the 21-bromo-3(α)-hydroxy derivative. With acetic acid and sodium acetate the 21-acetoxy group was restored, and a single oxidation with chromic acid then yielded the 3,11,20-triketo-21-acetoxy compound.

It should be noted that the introduction of oxygen at C_{11} as the first step and subsequent elaboration of the ketol at C_{20}:C_{21} from the choladiene derivative completes three of the four changes with a minimal amount of labor, and the excellent yields indicate that it will be difficult to secure significant improvement for these three steps.

The Unsaturated Ketone in Ring A. For the partial synthesis of the hormones of the adrenal cortex without oxygen at C_{11}, desoxycorticosterone, steroids such as cholesterol, stigmasterol, or other starting material with a double bond C_5:C_6 have been used. To change the double bond from its original position to C_4:C_5, it is necessary only to change the 3-hydroxyl group to a ketone. The double bond then shifts to form a conjugated group with the ketone in the A ring.[23] In the partial synthesis of the hormones with oxygen at C_{11}, it is necessary to start with a saturated bile acid and to introduce the double bond either in the A or in the B ring. Experience has shown that the best sequence for introduction of the double bond ketone in the A ring is after introduction of oxygen at C_{11} and elaboration of the ketol at C_{20}:C_{21}.

The simplest method to introduce the double bond is to substitute an atom of hydrogen at C_4 with bromine and then to remove hydrogen bromide.[24] Bromination at C_4 can be carried out with satisfactory yields, but unfortunately, removal of hydrogen bromide has not been accomplished with a correspondingly good yield.[2] At the present moment, this step in the partial synthesis is attended with loss of material which is greater than the loss at any other of the many steps. It seems highly probable that this modification in the structure of the steroid eventually will be accomplished in yields which are substantially higher than those which have been obtained in the past.

Introduction of Hydroxyl Group at C_{17}. Corticosterone and dehydrocorticosterone are examples of hormones of the adrenal cortex which have the ketol group at C_{20}:C_{21} and oxygen at C_{11}. During the past several years, evidence has accumulated that the physiologic action of corticosterone and dehydrocorticosterone is quantitatively inferior to that of the corresponding hormones which have in addition a hydroxyl group at C_{17}.

For the partial synthesis of hormones with a hydroxyl group at C_{17}, but without oxygen at C_{11}, von Euw and Reichstein[25] have prepared a 17,20,21,22-tetrahydroxy compound. The 21,22-acetone derivative of this was acetylated at 20 and, after removal of the acetone moiety, the 20,21-glycol was then converted with periodic acid to the 20-acetoxyl-21-aldehyde compound. Removal of the 20-acetyl group and treatment with pyridine brought about a rearrangement of the 20-hydroxy-21-aldehyde to the 21-hydroxy-20-ketone structure.

For the partial synthesis of 17-hydroxydehydrocorticosterone, Sarett[4] has devised a method which has as its essential step the preparation of a 17,20-dihydroxy-21-acetoxyl derivative through hydroxylation of 3(α),21-diacetoxy-11-keto-$\Delta^{17,\,20}$-pregnene. Oxidation with chromic acid yielded the 20-keto compound, but the yields of the intermediate compounds preclude the use of this method of synthesis for large-scale production.

The hormone with the remaining possible structure, that is, 17-hydroxycorticosterone (compound F), has not yet been partially synthesized.

The success which has attended efforts in the past is the best assurance that the remaining rough spots will be smoothed out and satisfactory methods for the commercial preparation of all of the hormones of the adrenal cortex will be devised.

It is interesting to note the progress which has been made since the partial synthesis of dehydrocorticosterone in 1943. The method of Lardon and Reichstein[2] required forty-one steps; the yield was approximately 0.04 per cent. Introduction of oxygen at C_{11} through the 3,9-epoxy compound, removal of the side chain, and elaboration of the ketol at $C_{20}:C_{21}$ through the diene reduced the number of steps to twenty-six and increased the yield, up to the introduction of the double bond $C_4:C_5$, to about 10 per cent. At the present time, the introduction of the double bond $C_4:C_5$ lowers the yield to between 3 and 4 per cent of that theoretically possible. Even with this great loss at the last step the improvement from the first synthesis is indeed striking.

Physiological Effects of the Synthetic Compounds. With the hormones at hand, what may be expected from their use in clinical medicine? I shall not venture a prediction, but shall point out that the critical condition of patients who have severe insufficiency of the hormones of the adrenal cortex can be controlled, if adequate amounts of the hormones are available. Furthermore, there is good evidence that under stress the adrenal cortex is stimulated to pour out its secretion. It does not seem probable that all patients are able to produce sufficient of the secretion at all times. If the hormones become plentiful enough to permit study of patients under conditions of stress, it may be possible to develop clinical tests to show that a deficiency exists and to treat patients who have latent or actual insufficiency.

About a year ago, Merck & Co., Inc., prepared a large sample of dehydrocorticosterone by the method which I have outlined for introduction of oxygen at C_{11} through the 3,9-epoxy structure. With experimental animals, it was shown by Drs. Dorfman, Venning, and Lewis that the crystalline hormone prepared by partial synthesis was identical with the natural product.* The identity of the chemical structure in the natural and partially synthesized products was beyond doubt. However, it was a matter of no little interest and importance to prove quantitatively that the biologic response to the two different preparations was the same.

Dehydrocorticosterone prepared by partial synthesis was used in the treatment of a series of patients who had Addison's disease.[26, 27] The results, however, were not striking. The reason for the failure to respond may be that there are differences between the experimental animal and the human being. The effect in rats on deposition of glycogen in the liver showed less than a one-fold difference when dehydrocorticosterone was used in place of 17-hydroxydehydrocorticosterone (compound E[28]). The protection of animals with compound A against cold and against the toxic effects of typhoid vaccine were notable.† However, in the patients who had Addison's disease, continued administration of large amounts of compound A did not produce a marked effect.[29]

It has already been shown that dehydrocorticosterone is much less toxic to normal mice than is compound E. To show how the mouse can tolerate compound A and how impossible it is to predict the physiologic effects of large amounts of these hormones, I shall report briefly some results obtained by Dr. Heilman and myself.[30] Pellets of crystalline compounds A, B, and E were given to four strains of mice over a period of four to five weeks. Under the conditions used, compound A did not cause death or lesions in the muscles. However, surprisingly large amounts of fat were deposited, even in young mice only 10 gm. in body weight. There was a slow increase of body weight with compound A. With compound B, there was a loss of weight, but still a marked increase of the deposition of fat. With compound E, there was more serious loss of body weight, and no deposition of fat and lesions were present in the muscles. The significance of these observations in the interpretation of the symptoms of patients who have hyperactivity of the adrenal cortex remains to be shown. It seems probable, however, that loss of muscle and deposition of fat in Cushing's disease may be related to the overproduction of compounds A, B, E, and F.

---

* These results were reported at a symposium held in Atlantic City, New Jersey, March 11, 1946.

† These results are given in the reports of Drs. Dorfman, Venning and Lewis which follow.

My chief interest for the past six years in the problems associated with the adrenal cortex has been to make the active compounds available for use. In this phase of the problem, it is a matter of satisfaction to be able to report the progress which has been made through the use of synthetic organic chemistry as a tool for the investigation of problems in normal physiology and clinical medicine.

Bibliography

1. Steiger, M., & T. Reichstein. 1937. Desoxy-cortico-steron (21-Oxy-progesteron) aus Δ^5-3-Oxy-ätio-cholensäure (XII. Mitteilung über Bestandteile der Nebennieren-Rinde). Helv. Chim. Acta **20:** 1164–1179.
2. Lardon, A., & T. Reichstein. 1943. Über Bestandteile der Nebennierenrinde und verwandte Stoffe; Teilsynthese des 11-Dehydro-corticosterons. Helv. Chim. Acta **26:** 747–755.
3. von Euw, J., A. Lardon, & T. Reichstein. 1944. Über Bestandteile der Nebennierenrinde und verwandte Stoffe; Teilsynthese des Corticosterons. Helv. Chim. Acta **27:** 1287–1296.
4. Sarett, L. H. 1946. Partial synthesis of pregnene-4-triol-17(β), 20(β), 21-dione-3, 11 and pregnene-4-diol-17(β), 21-trione-3, 11, 20 monoacetate. J. Biol. Chem. **162:** 601–631.
5. Lardon, A., P. Grandjean, J. Press, H. Reich, & T. Reichstein. 1942. Über Gallensäuren und verwandte Stoffe. Vereinfachte Herstellung von Cholen-(11)-säure-methylestern durch thermische Spaltung der 12-Benzoxy-Derivate. Helv. Chim. Acta **25:** 1444–1452.
6. Reich, H., & T. Reichstein. 1943. Über Gallensäuren und verwandte Stoffe. 11α-Keto- und 11α-Oxy-cholansäure. Helv. Chim. Acta **26:** 562–585.
7. Marker, R. E., & E. J. Lawson. 1938. Sterols. XXXIII. 3, 11-Dihydroxy-12-ketocholanic acid and derivatives. J. Am. Chem. Soc. **60:** 1334–1337.
8. Longwell, B. B., & O. Wintersteiner. 1940. The preparation and properties of 3(α), 11-dihydroxy-12-ketocholanic acid. J. Am. Chem. Soc. **62:** 200–203.
9. Gallagher, T. F. 1946. Partial synthesis of compounds related to adrenal cortical hormones; VI. The structure of the "3(α), 11-dihydroxy-12-ketocholanic acid" of Marker and Lawson and of the products obtained by "Wolff-Kishner reduction." J. Biol. Chem. **162:** 539–548.
10. Borgstrom, Evelyn, & T. F. Gallagher. 1946. Partial synthesis of compounds related to adrenal cortical hormones; VIII. Preparation of an 11-keto bile acid. J. Biol. Chem. **164:** 791.
11. Kendall, E. C. 1947. Steroids derived from the bile acids: 3, 9-epoxy-Δ^{11}-cholenic acid, an intermediate in the partial synthesis of dehydrocorticosterone. *Proceedings of the Laurentian Conference* **1:** 65–81. Academic Press Inc., New York.
12. Sarett, L. H. 1946. The structure of some derivatives of 3(α)-hydroxy-$\Delta^{9,11}$-cholenic acid. J. Biol. Chem. **162:** 591–600.
13. Turner, R. B., V. R. Mattox, L. L. Engel, B. F. McKenzie, & E. C. Kendall. 1946. Steroids derived from bile acids; III. Derivatives of $\Delta^{9,11}$-cholenic acid with substituents at C_3 and C_{12}. J. Biol. Chem. **162:** 571-584.
14. McKenzie, B. F., W. F. McGuckin, & E. C. Kendall. 1946. Steroids derived

from bile acids; I. The preparation of $3(\alpha)$-hydroxy-Δ^{11}-cholenic acid from desoxycholic acid. J. Biol. Chem. **162:** 555.

15. Engel, L. L., V. R. Mattox, B. F. McKenzie, W. F. McGuckin, & E. C. Kendall. 1946. Steroids derived from bile acids; II. $3(\alpha)$-hydroxy-11,12-dibromocholanic acid and related compounds. J. Biol. Chem. **162:** 565-570.
16. Mattox, V. R., R. B. Turner, L. L. Engel, B. F. McKenzie, W. F. McGuckin, & E. C. Kendall. 1946. Steroids derived from bile acids; IV. 3,9-epoxy-Δ^{11}-cholenic acid and closely related compounds. J. Biol. Chem. **164:** 569–596.
17. Turner, R. B., V. R. Mattox, L. L. Engel, B. F. McKenzie, & E. C. Kendall. 1946. Steroids derived from bile acids; V. Introduction of oxygen at C_{11}. J. Biol. Chem. **166:** 345–365.
18. Barbier, P., & R. Locquin. 1913. Method of decomposing various saturated mono- and dibasic acids. Compt. rend. Acad. sci. U.R.S.S. **156:** 1443–1446.
19. Wieland, Heinrich, Otto Schlichting, & Richard Jacobi. 1926. Untersuchungen über die Gallensäuren; über die Natur der Seitenkette und des vierten Ringes. Z. physiol. Chem. **161:** 80–115.
20. Kohler, E. P., & M. Tishler. 1932. The reaction between organic magnesium halides and alpha-bromo ketones. J. Am. Chem. Soc. **54:** 1594–1600.
21. Ziegler, K., A. Späth, E. Schaaf, W. Schumann, & E. Winkelman. 1942. Die Halogenierung ungesättigter Substanzen in der Allylstellung. Ann. Chem. **551:** 80–119.
22. Meystre, C., H. Frey, A. Wettstein, & K. Miescher. 1944. Über Steroide. Ein einfacher Abbau der Gallensäuren-Seitenkette zur Methylketonstufe. Helv. Chim. Acta **27:** 1815–1824.
23. Fernholz, E. 1934. Zur Synthese des Corpus-luteum-Hormons (Vorläuf. Mitteil.). Ber. Deutsch. Chem. Gesellsch. **67:** 1855.
24. Butenandt, Adolf, & Josef Schmidt. 1934. Über Δ^4-Pregnenol-(20)-on-(3); ein Beitrag zur Spezifität der Corpus-luteum-Wirkung. Ber. Deutsch Chem. Gesellsch. **67:** 2092–2094.
25. von Euw, J., & T. Reichstein. 1940. Über Bestandteile der Nebennierenrinde und verwandte Stoffe. Pregnen-(4)-diol-(17,20)-on-(3)-al-(21)-monoacetat-(20). Helv. Chim. Acta **23:** 1114–1125.
26. Thorn, G. W., P. H. Forsham, F. T. Garnet Prunty, G. E. Bergner, & A. Gorman Hills. 1949. Clinical Studies in Addison's Disease. Ann. N. Y. Acad. Sci. **50** (6): 646–656
27. Sprague, R. G., C. F. Gastineau, H. L. Mason, & M. H. Power. 1948. Effects of synthetic 11-dehydrocorticosterone (Compound A) in a subject with Addison's disease. Am. J. Med. **4:** 175–185.
28. Reinecke, R. M., & E. C. Kendall. 1943. Comparison of influence of some crystalline hormones of adrenal cortex on deposition of glycogen in liver. Endocrinology **32:** 505–508.
29. Sprague, R. G., E. J. Kepler, F. R. Keating, & M. H. Power. Unpublished data.
30. Kendall, E. C. 1945. Influence of the adrenals on carbohydrate metabolism. In Reifenstein, E. C., Jr.: Conference on metabolic aspects of convalescence including bone and wound healing: 81–97. Josiah Macy, Jr. Foundation, New York.

APPENDIX

(The following three papers constitute the first demonstration that the biological properties of synthetic and natural 11-dehydrocorticosterone are identical.)

1. STUDIES ON THE PROTECTIVE POWER OF ADRENAL EXTRACT AND STEROIDS AGAINST BACTERIAL TOXINS IN ADRENALECTOMIZED RATS

By Lena A. Lewis and Irvine H. Page
Research Division of the Cleveland Clinic Foundation, Cleveland, Ohio

It has been shown that the protective power of adrenal extracts or steroids against the acute toxic effect of typhoid vaccine can be accurately measured by testing the material on adrenalectomized rats.[1]

The assay is carried out on male Sprague-Dawley rats weighing between 80 and 120 gms., which are adrenalectomized under ether anesthesia, and maintained on 0.9 per cent sodium chloride drinking water for at least 5 days before starting the test. The animals are injected subcutaneously, once daily, if the hormone is in oil solution, twice daily, if in aqueous or alcohol-aqueous solution. Immediately after the third morning injection of hormone, 1.33 minimal lethal doses (M.L.D.) of typhoid vaccine are administered intraperitoneally. The M.L.D. is determined previously on saline-maintained adrenalectomized rats. The percentage of rats surviving 24 hours after receiving 1.33 M.L.D. typhoid vaccine is noted. One toxic protection unit, as we have defined it, is the amount of material, in terms of any convenient unit, per 24 hours, which will protect 90 per cent of adrenalectomized rats against 1.33 M.L.D. of typhoid vaccine and is equivalent in protective power to 0.28 mg. 11-dehydrocorticosterone acetate.

A series of experiments were carried out using 11-dehydrocorticosterone (Compound A) and 11-dehydrocorticosterone acetate prepared by isolation from adrenal tissue, 11-dehydrocorticosterone acetate prepared by partial synthesis,[3] and Compound E. The results obtained on the 4 materials are summarized in FIGURE 1. The natural and synthetic Compound A acetate,

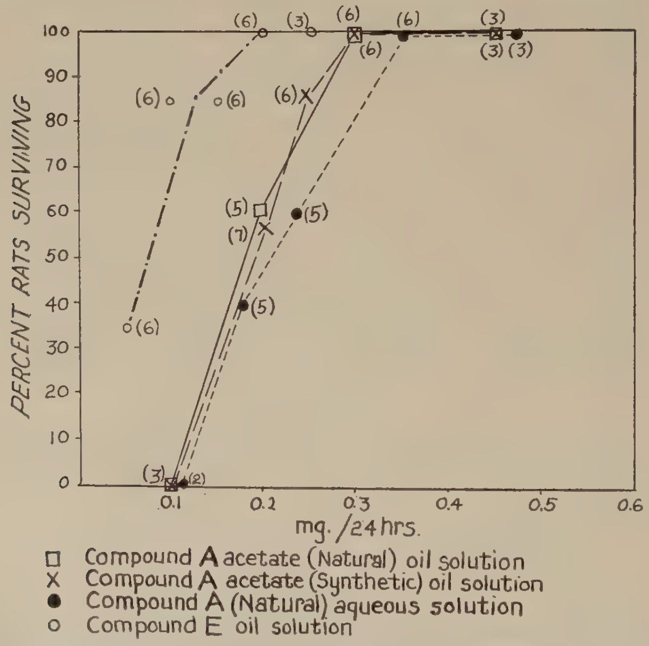


FIGURE 1. Comparison of protective power of natural and synthetic Compound A Acetate, Compound A, and Compound E against typhoid vaccine in adrenalectomized rats.

when administered in oil, gave nearly identical results. The natural Compound A in aqueous solution appeared to be slightly less effective. Ninety per cent of the rats were protected by 0.28 mg. of the Compound A acetate. Compound E was the most active of the materials tested.

Blood leucocyte counts and differential blood counts were made on adrenalectomized rats which were maintained on saline, or treated with whole adrenal extract, or with synthetic Compound A acetate before, and 2 hours after, the intraperitoneal injection of 1.33 M.L.D. of typhoid vaccine. TABLE 1 summarizes the results obtained. In the extract-treated and Compound A acetate-treated rats, the total leucocyte count showed no significant change 2 hours following administration of toxin. The lymphocytes decreased 7.8 thousand per cu. mm. and 8.9 thousand per cu. mm. respectively,

TABLE 1
ADRENALECTOMIZED RATS

	W.B.C. 1000/ cmm.	Per cent lymphocytes	Lymphocytes 1000/ cmm.	Per cent neutrophiles	Neutrophiles 1000/ cmm.
Extract treated					
Before typhoid Average 13 animals range	28.9 (23.25–41.75)	77.6 (69–88)	22.4	19.4 (8–29)	5.8
Two hours after typhoid Average 13 animals range	29.7 (22.0–42.0)	49.2 (29–67)	14.6	46.2 (31–69)	13.7
Change	+0.8		−7.8		+7.9
Saline treated					
Before typhoid 10 animals	31.7 (22.9–50.5)	81.4 (72.88)	25.8	15.2 (6–24)	4.8
Two hours after typhoid	27.5 (20.1–36.8)	64.4 (45–87)	17.7	30.0 (7–48)	8.4
Change	−4.2		−8.1		+3.4
Compound A treated					
Before typhoid 15 animals	26.58 (18.1–31.6)	73.8 (53–93)	19.6	26.3 (7–45)	7.0
Two hours after typhoid	26.35	40.7 (21–61)	10.7	58.1 (28–72)	15.3
Change	+1.3		−8.9		+8.3
Saline treated					
Before typhoid 13 animals	29.8 (17.3–46.1)	79.9 (59–89)	23.8	18.8 (9–40)	5.6
Two hours after typhoid	22.2 (13.7–31.2)	54.4 (38–73)	12.1	44.4 (26–56)	9.8
Change	−7.6		−11.7		+4.2

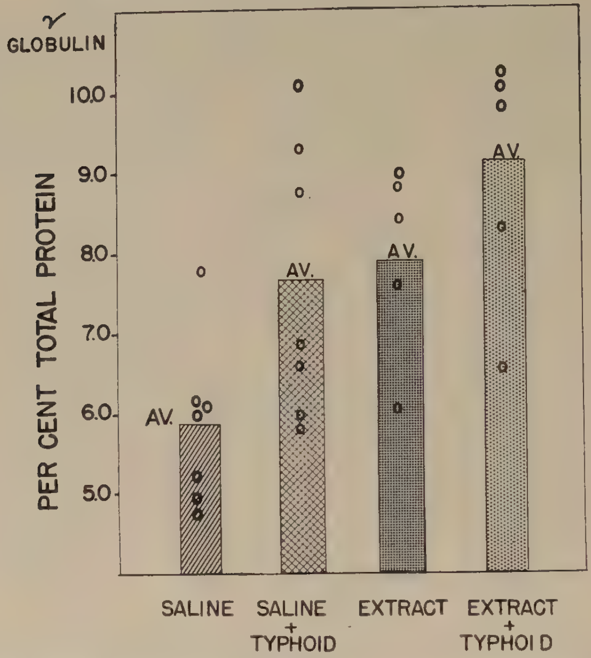


Figure 2. The γ-globulin concentration of serum of adrenalectomized rats treated with saline, or with adrenal extract before and 2 hours after administration of typhoid vaccine.

while the polymorphonuclear neutrophiles increased 7.9 and 8.3 thousand per cu. mm. respectively. In contrast with the hormone-treated groups, the animals receiving no replacement therapy, except saline, showed a decrease in the total leucocyte count. The lymphocyte count decreased to about the same extent as that of the other groups, but the neutrophiles showed a very much smaller increase. It is interesting that the changes in the blood picture are similar, whether the animal is treated with natural hormones or with synthetic Compound A acetate.

Whole adrenal extract and compound A acetate, also, have power to protect adrenalectomized rats against lethal doses of diphtheria toxin. Relatively larger amounts of hormone are required to protect against 1.25 M.L.D. of diphtheria toxin than against 1.33 M.L.D. typhoid toxin.

While the protective power of the various hormone preparations tested against typhoid toxin closely parallels their effect on carbohydrate metabolism, the maintenance of a normal blood sugar level is not alone capable of protecting the rats against the toxin.[1]

In an attempt to elucidate further the mechanism of the protective action of adrenal extract and steroids against typhoid toxin, serum proteins of adrenalectomized rats maintained on saline and of animals treated with whole adrenal extract were studied by the Tiselius electrophoresis method. Determinations were made on other similarly prepared animals 2 hours following the intraperitoneal injection of 1.33 M.L.D. typhoid vaccine. The average γ-globulin level of the extract-treated group was somewhat higher than the saline-treated animals, being 5.9 and 8.0 per cent respectively.

Following typhoid vaccine, the percentages increased in both groups, being 7.7 and 9.1 per cent respectively, 2 hours after administration of toxin. The increase in the γ-globulin is of interest in view of the fall in the lymphocyte counts. It has been shown by Dougherty and White[2] that the dissolution of lymphocytes is accompanied by an increase in γ-globulin of the serum.

Summary

The toxic protective power in adrenalectomized rats of natural Compound A acetate and of Compound A acetate prepared by partial synthesis against 1.33 M.L.D. typhoid vaccine is identical. Compound A acetate and adrenal extract-treated adrenalectomized rats show a greater increase in blood polymorphonuclearneutrophiles 2 hours following typhoid vaccine administration than do saline-treated controls 2 hours after toxin. A similar decrease in blood lymphocytes is observed in all groups.

The serum γ-globulin of extract-treated adrenalectomized rats is somewhat higher than that of saline-treated animals. Both groups show an increase in the γ-globulin level 2 hours after typhoid toxin administration.

References

1. Lewis, L. A., & I. H. Page. 1946. J. Lab. Clin. Med. **31:** 1325.
2. White, A., & T. F. Dougherty. 1944. Abs. Proc. Meetings Am. Chem. Soc. New York.
3. Kendall, E. C. 1948. Ann. N. Y. Acad. Sci. **50**:(6): 540–547.

2. THE COMPARATIVE ACTIVITIES OF 11-DEHYDROCORTICOSTERONE ISOLATED FROM THE ADRENAL GLAND AND THAT PRODUCED SYNTHETICALLY

By Ralph I. Dorfman

Departments of Biochemistry and Medicine, Western Reserve University School of Medicine, and Lakeside Hospital, Cleveland, Ohio

This study is concerned with the biological evaluation of the adrenal cortical steroid 11-dehydrocorticosterone isolated from the gland and also realized by partial synthesis.[1] Since the synthetic material available at the time of this study was in the form of the acetate, the direct comparisons were made on this derivative, rather than on the free compound. The comparisons were made using two biological tests, the deposition of glycogen in the adrenalectomized mouse[2] and the increase in survival of the adrenalectomized rat subjected to a low environmental temperature.[3] A further comparison is reported of the relative activity of the free compound on the basis of the deposition of glycogen to that of the acetate.

The methods used are those reported in detail previously from this laboratory.

Comparison Using the Cold Test. Table 1 summarizes the results on the cold test. Both the compound from the gland and that from the partial synthesis were run simultaneously to increase the precision of the comparison. The material was administered at four different concentrations ranging from 40 micrograms to 320 micrograms. Calculation of the best fitting

TABLE 1

THE COMPARATIVE COLD TEST RESPONSE TO 11-DEHYDROCORTICOSTERONE ACETATE ISOLATED FROM THE ADRENAL GLAND AND THAT PRODUCED SYNTHETICALLY

Source of material	Amount administered	Number of adrenalectomized rats	Survival hours ± S.E.
	μg.		
0	0	21	9.4 ± 0.37
Synthetic	40	10	10.7 ± 0.88
	80	10	11.9 ± 0.97
	160	10	13.5 ± 1.17
	320	10	15.4 ± 0.74
Gland	40	10	9.8 ± 0.81
	80	10	11.3 ± 1.23
	160	10	11.9 ± 0.90
	320	11	14.2 ± 0.98

equations from the experimental data by the method of Fischer gave for the material from the gland

$$Y = 4.6\,x + 2.4,$$

and for the material from the synthesis

$$Y = 5.2\,x + 2.2.$$

The slopes of the curves were found not to be significantly different. The relative potency may be expressed as;

$$\frac{\text{Synthetic}}{\text{Gland}} = \frac{100}{60}.$$

At P = 0.05 the error range was from +85 to −46 per cent. Therefore, the difference was not significant.

Comparison Using Glycogen Deposition in the Rat. TABLE 2 summarizes the results on the glycogen deposition in the adrenalectomized mouse. The 11-dehydrocorticosterone acetate obtained from the gland was studied at three concentrations ranging from 50 to 200 micrograms, while the synthetic material was studied at four concentrations ranging from 50 to 400 micrograms per animal. The material isolated from the gland gave the following equation as calculated from the experimental data:

$$Y = 3.95\,x - 5.4.$$

The synthetic material gave the following equation:

$$Y = 7.00\,x - 10.7.$$

The slopes of the two experimentally derived lines did not differ significantly. The relative potency of the glandular and synthetic materials is expressed by the following relationship:

$$\frac{\text{Synthetic}}{\text{Gland}} = \frac{100}{66}.$$

Table 2

The Comparative Glycogen Response to 11-Dehydrocorticosterone Acetate Isolated from the Gland and That Produced Synthetically

Source of material	Amount administered	Number of animals	Mg. of glycogen per 10g. of B.W. ± S.E.
	μg.		
0	0	0	0.5 ± 0.2
Synthetic	50	7	1.1 ± 0.8
	100	14	3.3 ± 0.8
	200	12	5.6 ± 0.8
	400	14	7.4 ± 0.5
Glandular	50	8	1.0 ± 0.5
	100	13	2.8 ± 0.9
	200	14	3.5 ± 0.5

Calculation of the error of the potency ratio gave the limits at $P = 0.05$ of +43 to −30 per cent.

The 11-dehydrocorticosterone was compared to that of the acetate by using the data previously reported for the free compound. On a molar basis, the following relative activity of the free compound and acetate was found to be:

$$\frac{\text{Free Compound}}{\text{Acetate}} = \frac{100}{63}.$$

The error range of the potency ratio was found to be +29 to −22 per cent.

Thus, there appears to be no difference between the material isolated from the gland and that prepared by partial synthesis, when tested by either the cold test or by a method involving the carbohydrate activity. On a molar basis, the free compound appears to be more active than the acetate when tested by a glycogen deposition method in the mouse.

References

1. Kendall, E. C. 1948. N. Y. Acad. Sci. **50**(6): 540–547.
2. Dorfman, R. I., E. Ross, & R. A. Shipley. 1946. Endocrinology **38**: 178.
3. Dorfman, R. I., R. A. Shipley, S. Schiller, & B. N. Horwitt. 1946. Endocrinology **38**: 165.

3. BIOLOGICAL ACTIVITY OF SYNTHETIC 11-DEHYDROCORTICOSTERONE ACETATE

By Eleanor H. Venning

McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

The biological activity of 11-dehydrocorticosterone acetate prepared by partial synthesis[1] was determined by the bioassay method of Venning, Kazmin, and Bell.[2] This method is based upon the ability of adrenal steroids to cause an increase in liver glycogen in fasted adrenalectomized mice. The animals are fasted for 16 hours prior to the assay, and the extract to be tested is administered in 7 divided doses, over a period of $5\frac{1}{2}$ hours. Ten mg. glucose are given with each injection. This small amount of glucose fails to cause any significant increase in the liver glycogen of the control adrenalectomized mice, but has the effect of greatly increasing the sensitivity of the method. One hour following the last injection, the livers are quickly removed and the glycogen content determined. The amount of liver glycogen is compared with that deposited by a standard adrenal cortical compound.

The biological activity of the synthetic preparation has been compared with the natural 11-dehydrocorticosterone acetate derived from the gland and also with the free crystalline compounds, 11-dehydrocorticosterone and 17-hydroxy 11-dehydrocorticosterone.

The compounds were assayed at two dose levels, 40 μg. and 80 μg. respectively. Duplicate assays were carried out using a total of 8–9 mice per assay. In FIGURE 1, the logarithm dose-response regression lines of the various compounds are shown. At a dose level of 80 μg., synthetic 11-dehydrocorticosterone acetate caused a liver glycogen deposition of 63 mg. per 100 gm. body weight, 11-dehydrocorticosterone acetate (natural product from the adrenal gland), a deposition of 69 mg. per 100 gm. body weight, and the free compound 11-dehydrocorticosterone, a deposition of 70 mg. per 100 gm. body weight. Thus, the capacity of the synthetic compound to cause a deposition of liver glycogen is only slightly lower than that of the acetate derived from the adrenal gland. Acetylation of 11-dehydrocorticosterone apparently does not interfere with the biological activity of the hormone.

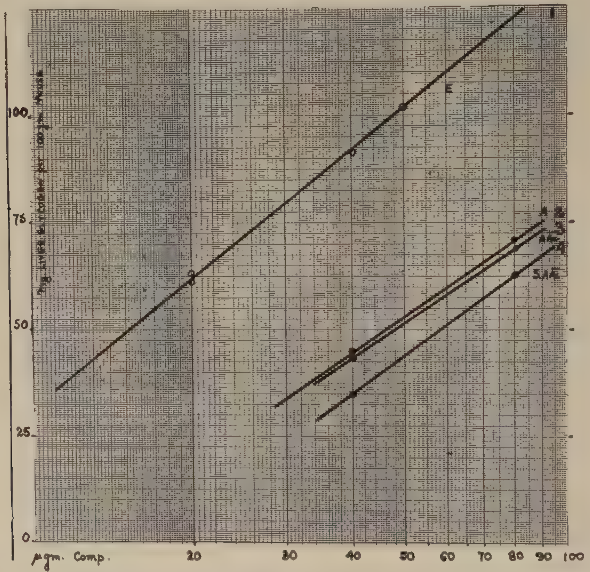


FIGURE 1. Logarithm dose-response regression lines. (1) 17-OH 11-dehydrocorticosterone, (2) 11-dehydrocorticosterone (3) 11-dehydrocorticosterone acetate (4) synthetic 11-dehydrocorticosterone acetate.

When the activity of 11-dehydrocorticosterone was compared with that of 17-hydroxy 11-dehydrocorticosterone, it was found that only 24 μg. of the latter compound were required to cause a deposition of liver glycogen of 70 mg. per 100 gm. body weight. By this method of assay, 17-hydroxy-11-dehydrocorticosterone is over three times more active in its effect on liver glycogen deposition than 11-dehydrocorticosterone.

References

1. KENDALL, E. C. 1948. Ann. N. Y. Acad. Sci. **50**(6): 540–547.
2. VENNING, E. H., V. E. KAZMIN, & J. C. BELL: 1946. Endocrinology **38:** 79.

THE BIOASSAY OF ADRENAL CORTICAL STEROIDS

By Ralph I. Dorfman

Departments of Medicine and Biochemistry, Western Reserve University School of Medicine, and Lakeside Hospital, Cleveland, Ohio

This paper is concerned with the evaluation of methods for the quantitative assay of the adrenal cortical activity of pure compounds, glandular extracts and urinary extracts. A relatively large number of methods have been suggested for this purpose, but only few have been studied in detail, and even fewer have been submitted to statistical analysis to ascertain the errors of estimation. It is the purpose of this summary to deal critically with representative methods which have been used. The methods will be discussed from the standpoint of sensitivity and reproducibility. No attempt will be made to evaluate all the variables in any one test; these details can be found in the original papers.

As a preliminary to the discussion of the methods for the assay of adrenal cortical material, it may be well to deal briefly with four topics which will help to define our problem. These topics are: (1) the role of the adrenal cortex in the body economy; (2) the biologically active substances or hormones which have been isolated from the adrenal cortex; (3) a summary of the various types of bioassays that have been suggested; and (4) a brief description of the statistical methods employed for data analysis.

Role of the Adrenal Cortex

The physiological action of the adrenal cortical hormones has been excellently summarized by Ingle.[11] It is sufficient, for the purposes of this paper, to set forth some of the more important biological actions of the adrenal cortical hormones. The bulk of our knowledge of the biological action of these hormones has come from studies involving the removal of the adrenal cortex either from experimental animals or from the Addison's disease patient. Animals deprived of adrenal cortical hormones usually die in varying lengths of time, depending upon the species and age of the animal. The diet, particularly the concentrations of sodium and potassium, determine the survival time of the operated animals. Adrenalectomized animals are sensitive to all forms of stress such as changes in environmental temperature, external pressure, toxins, chemical poisons, infections, *etc.*

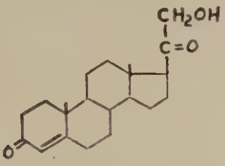
Adrenalectomy results in certain digestive disturbances such as loss of appetite, poor gastro-intestinal absorption, and diarrhea. The last symptom is particularly marked in advanced adrenal insufficiency.

Specific alterations in carbohydrate and mineral metabolism have been observed. Thus, in the absence of the adrenal cortex the animal appears to have difficulty in converting proteins to carbohydrates and tends to excrete excessive quantities of sodium and decreased quantities of potassium.

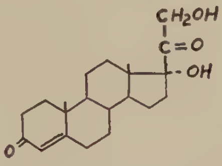
White and Dougherty[20] have demonstrated an influence of adrenal cortical steroids on protein metabolism. Essentially these workers have demonstrated that adrenal cortical hormones cause dissolution of lymphoid tissue, a decrease in the total leucocyte count, a decrease in the absolute numbers of lymphocytes, and an increase in the absolute number of polymorphonuclear cells. Simultaneously, there is a rise in the total serum protein. The increase in plasma proteins appears in the globulin fraction.

Other changes in the adrenalectomized animal include such circulatory changes as hemoconcentration, decreased blood pressure, and blood flow.

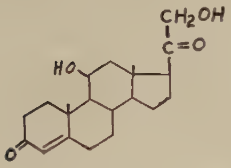
Hormones Isolated from the Gland. All the symptoms which result from adrenal cortical insufficiency may be reversed by the administration of suitable extracts of the adrenal cortex. From these extracts, six well-defined compounds having adrenal cortical activity have been isolated and characterized.[17] Some of these compounds appear to be capable of correcting all of the symptoms, while others have more limited biological activity. The six compounds are illustrated in FIGURE 1. Four of the compounds, corticosterone, 17-hydroxycorticosterone, 11-dehydrocorticosterone, and 17-hydroxy-11-dehydrocorticosterone, are characterized as having oxygen on carbon atom 11, either as an hydroxyl group or as a carbonyl group, while two of the compounds do not have oxygen at carbon 11. This is of interest, particularly, from the standpoint of carbohydrate metabolism, since the oxygen at carbon 11 appears to be essential for this activity. This point will be more clearly demonstrated later when we discuss the relative activi-



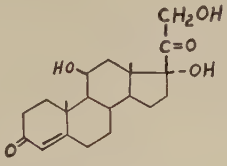
11-Desoxycorticosterone



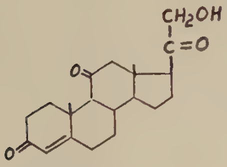
17-Hydroxy-11-desoxycorticosterone



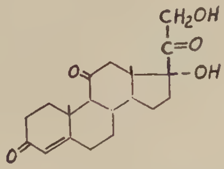
Corticosterone



17-Hydroxycorticosterone



11-Dehydrocorticosterone



17-Hydroxy-11-dehydrocorticosterone

FIGURE 1. Steroids isolated from the adrenal cortex possessing biological activity.

ties of the pure compounds. In addition to the six compounds mentioned, some twenty-odd other crystalline steroids have been isolated and identified. Some of these compounds have been shown to be inactive while others have not been adequately studied.

Suggested Methods for Bioassay of Adrenal Cortical Hormones. The bioassay methods suggested in adrenalectomized animals may be classified as those dealing with growth and survival, those dealing with glycogen deposition in the liver of fasting animals or related effects on carbohydrate metabolism, and those dealing with various forms of stress and miscellaneous bioassays. It is fully realized that in some instances there exists overlapping as to type of test represented, and the classification used here is merely one of convenience.

Survival-Growth Methods. The adrenalectomized dog, cat, rat, mouse, drake, and guinea pig have been employed from time to time as test animals. Such end points as growth of the adrenalectomized animal, the length of survival, or percentage survival have been employed. One important factor is diet, since a diet rich in sodium and poor in potassium is known to lead to indefinite survivals in the absence of undue stress or infections. Some preliminary evaluations of a growth-survival method using the rat will be presented.

Bülbring[1] described a method using the survival time of the adrenalectomized drake. The method had the advantage of being rapid, but, since the animals died in a matter of hours, the question arises as to the importance of the factor of stress. Thus, the test may measure the capacity of protecting animals against stress, as well as survival. The error of the potency ratio using relatively small numbers of animals (total of 20) appeared to be in the range of 33 per cent at $P = 0.05$. The method appears to be relatively sensitive, but has not been applied to crystalline material, so that it is difficult to get a measure of its relative sensitivity.

POTASSIUM METABOLISM. For studies on electrolyte metabolism, the potassium intoxication test has been suggested but not developed into an adequate bioassay due to the fact that the variation in sensitivity of adrenalectomized animals to potassium is great. In controlled experiments using adrenalectomized rats, it has been possible to detect about 0.75 mg. of desoxycorticosterone acetate per animal with a potassium intoxication test.[6] The accuracy of the assay using 20 animals on a standard and 20 on an unknown would probably not be any greater than ±50 per cent, but not enough data is available to evaluate the data with certainty.

Sodium Metabolism. Although a number of methods have been described dealing with sodium excretion, no statistical evaluation of the methods are available. In a recent study,[13] normal dogs were employed and it was found that about 0.7 mg. of desoxycorticosterone acetate could be detected, but the error of estimation was not evaluated. Preliminary studies in the author's laboratory on rats, using radiosodium, have indicated a relatively sensitive and reproducible method, which is being studied at the present time.

Carbohydrate Metabolism. In the category of carbohydrate metabolism, there are methods dealing with the deposition of glycogen in the liver of the fasting adrenalectomized rat and mouse, the work test of Ingle, and the anti-insulin action of adrenal cortical material. With the exception of the last test, detailed statistical summaries are included. The anti-insulin test has been found to detect 0.5 mg. of corticosterone, 17-hydroxycorticosterone, and 17-hydroxy-11-dehydrocorticosterone.[9] The sensitivity is perhaps greater than 0.5 mg. per animal, but the variability has been found to be great in some unpublished work in the author's laboratory.

Stress Tests. Among the stress tests for adrenal cortical material, there are such tests as exposure to low environmental temperatures, histamine, typhoid toxin, and the effect of restraining animals. Of the various methods, only the cold test has been subjected to statistical anaylsis and these studies will be discussed in some detail.

Statistical Methods

The various data discussed in this paper have been submitted to statistical analysis by known reliable methods. A brief description of these methods is in order.

Standard deviations have been calculated by the following formula:

$$S.D. = \sqrt{\frac{\Sigma(d)^2}{n-1}}$$

where $S.D.$ = Standard Deviation

d = Individual Deviations from the Mean
n = number of observations.

Standard error of the mean has been calculated by the following formula:

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

where $S.E.$ = Standard Error of the Mean.

The regression coefficients have been calculated by the method of Fischer,[8] according to the following formula:

$$Y = a + b\,(x - \bar{x})$$

where b = slope $= \dfrac{\Sigma y(x-\bar{x})}{\Sigma(x-\bar{x})^2}$

$a = \bar{y}$ = mean of y
$\bar{x}$ = mean of x.

The relative potencies have been determined by the method of Irwin[12] by the following formula, after proving that the slopes of the regression lines are not significantly different:

$$[\log \text{potency ratio} = \bar{x}_s - \bar{x}_u + \frac{\bar{y}_u - \bar{y}_s}{b_c}$$

where $b_c = \dfrac{[\Sigma y(x-\bar{x})]_s + [\Sigma y(x-\bar{x})]_u}{[\Sigma(x-x)^2]_s + [\Sigma(x-\bar{x})^2]_u}$

$\bar{x}_s$ = mean of x of standard
$\bar{x}_u$ = mean of x of unknown
$\bar{y}_s$ = mean of y of standard
$\bar{y}_u$ = mean of y of unknown.

The error of the potency ratio was calculated by the following formula:

$$\lambda = \frac{S.D.}{b_c}\sqrt{\frac{1}{m_s}+\frac{1}{m_u}}$$

where $S.D.$ = weighted mean standard deviation.

The antilog of 2 times λ gives the high limit of error at $P = 0.05$ above the value determined, while the antilog of the reciprocal gives the lower limit at $P = 0.05$.

The method of Bliss has been employed in a few instances and has been described in detail.[2] The experimental design consists of two concentrations each of the unknown and standard, so that the following proportion holds: $\frac{U_1}{U_2} = \frac{S_1}{S_2}$. From the individual observations, the slope, the relative potency, the error of the relative potency, and the deviation from parallelism of the two lines may be calculated. This latter step is essential, since the comparison is not valid unless the slopes of the lines are not significantly different.

Analysis of a Growth-Survival Method in Adrenalectomized Rats

For the analysis of this method, some data* dealing with the survival and growth of rats adrenalectomized at 28 days of age were employed. The operated animals were treated for 20 days with either crystalline adrenal cortical steroids or adrenal cortical extracts. The results of the statistical evaluation are presented in TABLE 1 and summarize the relative potency of the three adrenal cortical steroids, 17-hydroxy-11-dehydrocorticosterone, 17-hydroxycorticosterone, and corticosterone. Calculations on an extract of the adrenal cortex is included to indicate the fact that the slope is essentially similar to that of 17-hydroxy-11-dehydrocorticosterone. In TABLE 2,

TABLE 1

THE RELATIVE POTENCY OF ADRENAL CORTICAL STEROIDS AND AN ADRENAL EXTRACT ON THE BASIS OF A GROWTH METHOD (20 DAY TEST)*

Material	Number of animals	Slope	Relative potency	Error ($P = 0.05$) %	“p”
17-Hydroxy 11-dehydrocorticosterone	26	28.2	100	—	—
17-Hydroxy-corticosterone	18	43.2	219	−34, +51	0.365
Corticosterone	14	49.6	108	−32, +47	1.263
Lipo-Adrenal L-7234-35	29	39.5	—	—	0.788

* From data kindly submitted by Dr. M. H. Kuizenga.

---

* These data were kindly made available to me by Dr. Kuizenga of the Upjohn Company.

TABLE 2

RELATIONSHIP BETWEEN SURVIVAL AND GROWTH OF THE ADRENALECTOMIZED RAT TREATED WITH ADRENAL CORTICAL STEROIDS AND EXTRACT*

Material administered	Amount of material administered per rat per day	Mean weight gain in 20 days	Fraction of rats surviving 20 days
	μg.		
Lipo Extract	0.1 (W.U.)†	32.9	10/20
17-Hydroxy 11-dehydro-corticosterone	100	18.9	9/9
	156	25.8	5/5
17-Hydroxy-corticosterone	100	27.8	9/13
	200	41.6	10/10
Corticosterone	100	15.6	7/10
	250	36.0	9/10

* From data kindly submitted by Dr. M. H. Kuizenga.
† W. U. = Work Unit, an arbitrary unit.

we have an indication that there is not necessarily a direct relationship between the absolute weight gain of a treated adrenalectomized animal and its survival. Thus, at a time when some animals treated with Lipo-extract averaged gains of 32.9 grams in a 20 day period, only 10 of the 20 animals in the group survived 20 days, while similar animals treated with 17-hydroxy-11-dehydrocorticosterone showed gains of only 18.9 grams in 20 days, but 100 per cent survival. These illustrations are only to be taken as preliminary indications and the point should be submitted to further study.

Analysis of Methods Dealing with Carbohydrate Metabolism

Use of the Adrenalectomized Rat. The deposition of glycogen in the fasting adrenalectomized rat is the most precise method available for the bioassay of adrenal cortical material but not the most sensitive. Since the introduction of this method by Reinecke and Kendall,[18] it has been used widely. In this paper, the data of Pabst *et al.*[15] and Olson *et al.*[14] are considered in detail.

The dosage range employed by Pabst *et al.*[15] is illustrated in TABLE 3.

TABLE 3

QUANTITIES OF ADRENAL CORTICAL STEROIDS USED FOR STUDIES ON RELATIVE POTENCIES*

Compound	Amount of steroid per rat
	μg.
17-Hydroxy-11-dehydrocorticosterone	250 to 1000
Corticosterosterone	350 to 1000
17-Hydroxvcorticosterone	125 to 1000
11-Dehydrocorticosterone	350 to 1000

* Pabst *et al.*[15]

The minimum amount of material that could be used per animal is in the range of 250 micrograms equivalent of 17-hydroxy-11-dehydrocorticosterone.

TABLE 4 illustrates the utility of the method when relatively large num-

TABLE 4

THE RELATIVE ACTIVITIES OF VARIOUS ADRENAL CORTICAL STEROIDS EXPRESSED IN TERMS OF THE ACTIVITY OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE*

Compound	Number of rats	b ± S.E.	Potency ratio	Error (P = 0.05) %	"t"
17-Hydroxy-dehydrocorticosterone	50	1.848 ±0.191	100	—	—
Corticosterone	40	1.677 ±0.183	54	−13; +15	0.602
11-Dehydrocorticosterone	40	2.229 ±0.251	48	−10; +12	1.202
17-Hydroxycorticosterone	59	1.963 ±0.131	155	−12; +14	0.005

* Calculated from data of Pabst *et al.*[15]

bers of animals are employed. For each compound, between 40 and 59 animals were employed at various concentrations of the material. In each instance the slope of the compound studied did not differ significantly from the slope of the reference compound, 17-hydroxy-11-dehydrocorticosterone. The relationship considered was that of the logarithm of the dose and the response. It is seen that both corticosterone and 11-dehydrocorticosterone are less active than 17-hydroxy-11-dehydrocorticosterone, but do not differ in activity among themselves. 17-Hydrocorticosterone is significantly more active than 17-hydroxy-11-dehydrocorticosterone. Of interest is the low order of error of the potency ratio. The maximum errors found was −13 to +15 per cent at $P = 0.05$.

The utility of the method is again illustrated in TABLE 5, where the potency of hog and beef adrenal cortical extracts, on a weight basis, is presented in terms of 17-hydroxy-11-dehydrocorticosterone. A significantly greater activity was found for the hog extract and a low error in the potency ratio at $P = 0.05$, and no departure from parallelism of the respective slopes.

TABLE 5

THE ACTIVITY OF ADRENAL CORTICAL EXTRACTS IN TERMS OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE (COMPOUND E)*

Compound or extract	Number of animals	b ± S.E.	Grams of tissue equivalent to 1 mg. Compound *E*	Error (P = 0.05) %	"t"
17-Hydroxy-11-dehydrocorticosterone	50	1.848 ±0.191	—	—	—
Hog	39	2.244 ±0.187	93.4	+14; −12	1.760
Beef	37	2.134 ±0.467	214.1	+15; −13	0.608

* Calculated from data of Pabst *et al.*[15]

Of interest is the reproducibility of the method when smaller numbers of animals are employed. In TABLE 6 such results are illustrated. Again, 17-hydroxy-11-dehydrocorticosterone was employed as the reference compound, and in two comparisons a total of 40 animals were employed, while in four tests a total of 20 animals were used. The Bliss design[2] was used in these instances.

TABLE 6

THE RELATIVE POTENCY OF ADRENAL CORTICAL STEROIDS COMPARED TO 17-HYDROXY-11-DEHYDROCORTICOSTERONE*

Compound	Slope	Total number or animals	Potency ratio	Error ($P = 0.05$)	“*p*”
			%	%	
11-Hydroxycorticosterone	2.115	40	155	+29; −22	1.280
11-Dehydrocorticosterone	2.352	40	57.1	+25; −20	0.118
11-Hydroxycorticosterone	2.369	20	139	+34; −26	2.109
11-Hydroxycorticosterone	1.479	20	137	+96; −49	0.145
11-Dehydrocorticosterone	2.110	20	50	+48; −33	0.031
11-Dehydrocorticosterone	2.602	20	64.1	+30; −23	1.733

* Calculated from data of Pabst *et al.*[15]

In all comparisons, no departure from parallelism was detected. With a total of 40 animals at $P = 0.05$, an approximate error of ±24 per cent can be expected, while with the use of 20 animals an approximate error of ±43 per cent at $P = 0.05$ is indicated. With a total of 80 animals, an error of approximately ±15 per cent was found.

The data presented concerning the rat glycogen method thus far are those of Pabst *et al.*[15] It is of interest to compare these to the data of Olson *et al.*[14] The latter workers employed a similar method and their data were recalculated to conform with the calculations on the data of Pabst *et al.*[15] TABLE 7 illustrates the dosage range of the crystalline compounds used by Olson *et al.*[14] The calculations are presented in TABLE 8. The order of activity of the various compounds and the errors of the potency ratios agree rather well with those of Pabst *et al.*[15] However, the data on 17-hydroxycorticosterone could not be utilized, since the slope differed significantly from that of 17-hydroxy-11-dehydrocorticosterone.

TABLE 7

AMOUNTS OF CRYSTALLINE ADRENAL CORTICAL STEROIDS EMPLOYED BY OLSEN *et al.*[14]

Compound	Amounts of steroid used
	μg.
Corticosterone	470 to 1240
11-Dehydrocorticosterone	390 to 1740
17-Hydroxy-11-dehydrocorticosterone	330 to 1270
17-Hydroxycorticosterone	440 to 1080

Use of the Adrenalectomized Mouse. The rat glycogen method described has a relatively low order of error and has the further advantage that the

TABLE 8

THE RELATIVE ACTIVITIES OF ADRENAL CORTICAL COMPOUNDS EXPRESSED IN TERMS OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE*

Compound	Number of animals	b	Potency ratio	Error (P = 0.05)
				%
11-Dehydro-17-hydroxy-corticosterone	36	1.82	100	—
Corticosterone	23	1.88	75	−16 to +20
17-Hydroxycorticosterone	26	3.20	—	—
11-Dehydrocorticosterone	37	2.05	88	−13 to +16

* Calculated from data of Olsen *et al.*[14]

crystalline compounds and the extracts studied have similar slopes when considered as a logarithm dose-response relationship. However, the amount of material needed is rather large. In order to detect smaller amounts of material, particularly such as is needed for the determination of cortin-like material in urine, it became necessary to devise a more sensitive method. The mouse glycogen method is such a method and has been studied by Eggleston *et al.*,[6] Dorfman *et al.*,[3] and Venning *et al.*[19]

From the data in TABLE 9, which presents the regression coefficients, and FIGURE 2, which is the graphic representation of the logarithm of the dose-response relationship, it is seen that the slopes of such diverse materials as 11-dehydrocorticosterone, 17-hydroxy-11-dehydrocorticosterone, a hog adrenal cortical extract, and a urinary extract do not differ significantly.

TABLE 9

THE LINEAR REGRESSION FORMULAE OF 11-DEHYDROCORTICOSTERONE, 17-HYDROXY-11-DEHYDROCORTICOSTERONE, URINARY EXTRACT, AND PIG ADRENAL CORTICAL EXTRACT USING THE RELATIONSHIP BETWEEN LOGARITHM OF THE DOSE AND THE RESPONSE*

Material administered	Linear regression formulae	Remarks
11-Dehydrocorticosterone	$Y = 6.38x - 8.15$[1] $Y = 3.54 \pm 1.09 + 6.46 \pm 0.81 (x - \bar{x})$	
17-Hydroxy-11-dehydrocorticosterone	$Y = 22.23x - 27.36$ $Y = 7.05 \pm 0.74 + 22.23 \pm 3.08 (x - \bar{x})$	From 3 points, 20, 40, and 80 micrograms
	$Y = 6.66x - 4.81$	From 4 points, 5, 10, 20, and 40 micrograms
Urinary extract	$Y = 4.88x - 7.80$ $Y = 1.96 \pm 1.09 + 4.88 \pm 0.72 (x - \bar{x})$	
Pig adrenal cortical	$Y = 4.79x + 2.38$	From 4 points, 0.01, 0.02, 0.04, and 0.08 cc. $x = \log 100 \times$ dose

* From data of Dorfman *et al.*[3]

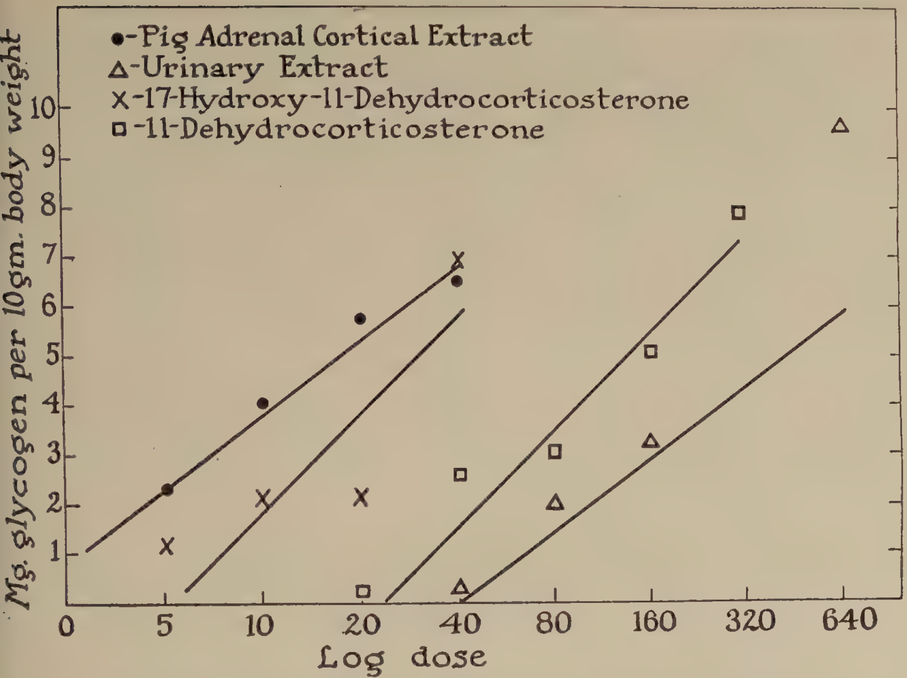


Figure 2. Liver glycogen responses to increasing doses of pig adrenal cortical extract, urinary extract, 17-hydroxy-11-dehydrocorticosterone, and 11-dehydrocorticosterone. Doses of pig adrenal cortical extract are plotted as the logarithm of 500 times the dose in cc. of oil solution. The urinary extract is plotted as the logarithm of the dose in cc. and the doses of the two adrenal cortical steroids are represented as the logarithm of the dose in micrograms.

Studies were conducted as to the error one may expect if approximately 5 or 10 animals are used on an unknown preparation and the values referred to a standard, in this case, 11-dehydrocorticosterone. This is of interest, since in normal human urine only enough material is available for the use of 5 to 10 animals when a 48-hour sample is used. The predictable error at $P = 0.05$ is -72 to $+260$ for 5 animals. In TABLE 10, it is seen that in 11 of 12 experiments (from 3 to 6 animals) the error ranged from 41 per cent low to 230 per cent high, and in one instance the value was 431 per cent high. In TABLE 11 a similar sort of analysis is presented for approximately 10 animals.[8–14] Here, the errors ranging from -50 to $+150$ were found in 16 experiments. The predictable error is actually -59 to $+150$ per cent when $P = 0.05$.

It was seen that a linear relation is apparent when the logarithm of the dose-response is plotted up to values of approximately 10 mg. of glycogen per 10 g. of body weight. However, if responses are considered up to approximately 20 mg. of glycogen per 10 g. of body weight for 17-hydroxy-11-dehydrocorticosterone and a pig adrenal cortical extract, the curve assumes an exponential character when plotted as logarithm of the dose *versus* response. If the data are reconsidered, plotted as the relationship between the logarithm of the dose and the logarithm of the response, a linear relationship is found. This is illustrated in FIGURE 3. Using this new relationship,

TABLE 10

VARIATIONS IN RESPONSE TO 80 MICROGRAMS OF 11-DEHYDROCORTICOSTERONE*
(Values read from standard 11-dehydrocorticosterone curve. Approximately 5 mice per group.)

Number of mice	Amount found: micrograms	Errors
		%
3	113	+41
4	49	−39
4	103	+29
4	105	+31
5	425	+431
5	100	+25
5	63	−21
5	265	+230
6	65	−19
6	130	+63
6	70	−13
6	70	−13

* From data of Dorfman *et al.*[3]

TABLE 11

VARIATIONS IN RESPONSE TO 80 MICROGRAMS OF 11-DEHYDROCORTICOSTERONE*
(Values read from standard 11-dehydrocorticosterone curve. Approximately 10 mice per group.)

Number of mice	Amount found: micrograms	Errors
		%
8	200	+150
8	40	−50
8	49	−39
10	44	−45
10	78	−3
10	130	+63
10	170	+113
10	130	+63
10	163	+138
10	160	+100
10	81	+1
11	118	+48
11	123	+54
11	83	+38
12	49	−39
14	60	−25

* From data of Dorfman *et al.*[3]

no significant difference was found in the slopes of the hog adrenal cortical extract and 17-hydroxy-11-dehydrocorticosterone up to a response of approximately 20 mg. of glycogen per 10 g. of body weight.

The relative activity of 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone was studied by Dorfman *et al.*[4] and by Venning *et al.*[19] These comparisons are tabulated in TABLE 12. The data of Venning *et al.*[19] have been recalculated by the statistical methods employed in the paper of Dorfman *et al.*[4] The method employed by Dorfman *et al.*,[4] and originally suggested by Eggleston *et al.*,[6] consisted in protecting the fall

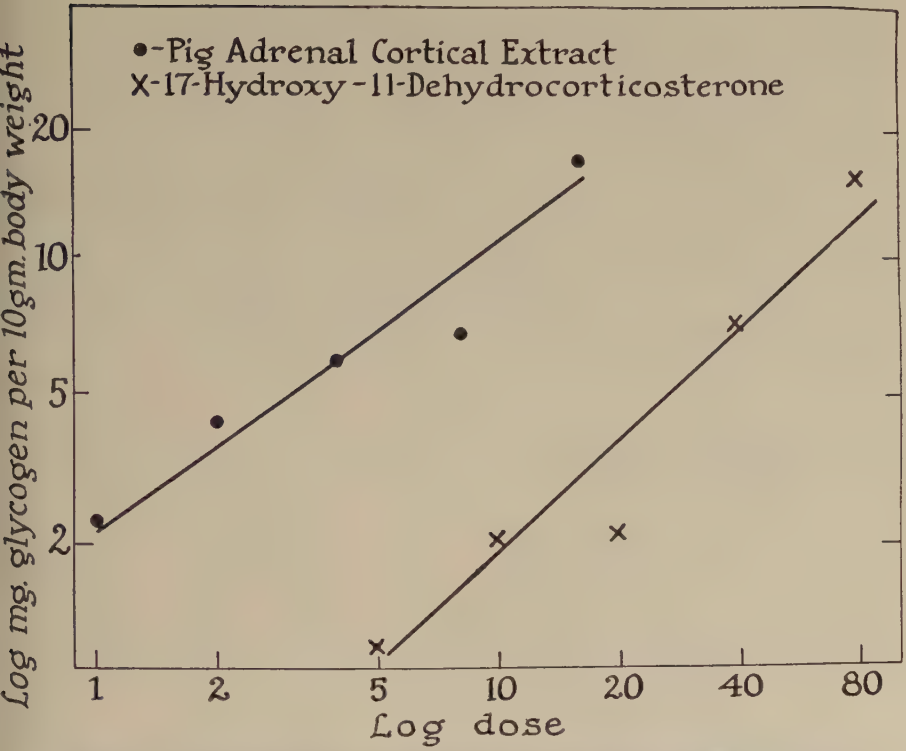


FIGURE 3. Liver glycogen responses to increasing doses of pig adrenal cortical extract and 17-hydroxy-11-dehydrocorticosterone. Doses of pig adrenal cortical extract are plotted as the logarithm of 100 times the dose in cc. of oil solution while doses of 17-hydroxy-11-dehydrocorticosterone are expressed as the logarithm of the weight in micrograms. The formula for 17-hydroxy-11-dehydrocorticosterone was $Y = 1.317\,x - 0.961$ and the formula for the pig adrenal cortical extract was $Y = 0.659\,x + 0.370$.

TABLE 12

COMPARISON OF THE ACTIVITIES OF 11-DEHYDROCORTICOSTERONE (A) AND 17-HYDROXY-11-DEHYDROCORTICOSTERONE (E) BY THE MOUSE GLYCOGEN METHOD

Method	Total number of animals	Potency of A in % of E	Error (P = 0.05)
			%
Protect fall of liver glycogen (Dorfman *et al.*)[4]	238	26	−25; +33
Build up liver glycogen (Venning *et al.*)[19]	73	29	−17; +20

in liver glycogen in a fasting adrenalectomized mouse, while the method of Venning *et al.*[19] consisted in building up the glycogen of the liver. Two points are of interest. First, it is seen that the relative potency of 11-dehydrocorticosterone and 17-hydroxy-11-dehydrocorticosterone by the two methods are in good agreement. Thus, 11-dehydrocorticosterone was 26 and 29 per cent as active as 17-hydroxy-11-dehydrocorticosterone by the methods of Dorfman *et al.*[4] and Venning *et al.*,[19] respectively. Secondly, the method of Venning *et al.*[19] appears to have the higher accuracy as seen by the fact that a lower error range of the potency ratio was found with fewer animals.

The sensitivity of the mouse glycogen method is in the range of 10 micrograms of 17-hydroxy-11-dehydrocorticosterone, which means the method is about 25 times as sensitive as the rat glycogen method.

The Ingle Work Test. When the Ingle work test is employed with the adrenalectomized-nephrectomized rat, the method appears to measure carbohydrate effects. When 15 animals are used on an unknown preparation and the values referred to 135 animals on the reference compound, accuracy is in the order of ±20.[15] The sensitivity of the method is in the range of 400 micrograms of 17-hydroxy-11-dehydrocorticosterone (TABLE 13).

TABLE 13

THE RELATIVE POTENCIES OF ADRENAL CORTICAL STEROIDS IN TERMS OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE ON THE BASIS OF THE WORK TEST*

Compound	Potency ratio 17-Hydroxy-11-dehydrocorticosterone taken as 100	Error ($P = 0.05$)
		%
17-Hydroxycorticosterone	192	±20
Corticosterone	63	
11-Dehydrocorticosterone	48	
11-Desoxycorticosterone	2	
17-Hydroxy-11-desoxycorticosterone	2	

* From data of Pabst *et al.*[15]

The Cold Test—An Example of a Stress Test. Beginning with the work of Hartman *et al.*,[10] the increased sensitivity of adrenalectomized animals to low environmental temperatures has been used as a means of determining adrenal cortical activity. A detailed description of the method has been published using operated mice and rats.[5] Since the rat was found to be the most reliable, our discussion will be confined to studies on the rat.

The test can be characterized as simple, rapid, and sensitive. Its usefulness is marred by an erratic variation in sensitivity of animals from group to group.

The use of the cold test can be illustrated by the assay of urinary extracts in terms of a crystalline standard, specifically 11-dehydrocorticosterone, and studies on the relative activities of various adrenal cortical steroids.

Some of the studies were done when the unknown and standard were run simultaneously, and in others, comparisons were made from group to group, only if two conditions were fulfilled in the various runs:

A. A minimum standard dose of 11-dehydrocorticosterone gave a statistically significant response.

B. The mean survival of the control animals did not differ significantly among the groups used.

Such rigid control was necessary, since in about one third of the runs the response was extremely low or zero. Although small amounts of urinary extract gave good responses in the cold test, it was found frequently that, after the maximum increase in survival time was attained, further increases in dosage caused a decreased response. Thus, in dealing with urinary extracts, it is necessary to be sure that one is working in the region of the ascending portion of the dose-response curve.

FIGURES 4, 5, and 6 illustrate the responses of 17-hydroxy-11-dehydrocorticosterone, corticosterone, and 11-desoxycorticosterone acetate, respectively, as compared to 11-dehydrocorticosterone. No significant differences were found in the respective slopes.

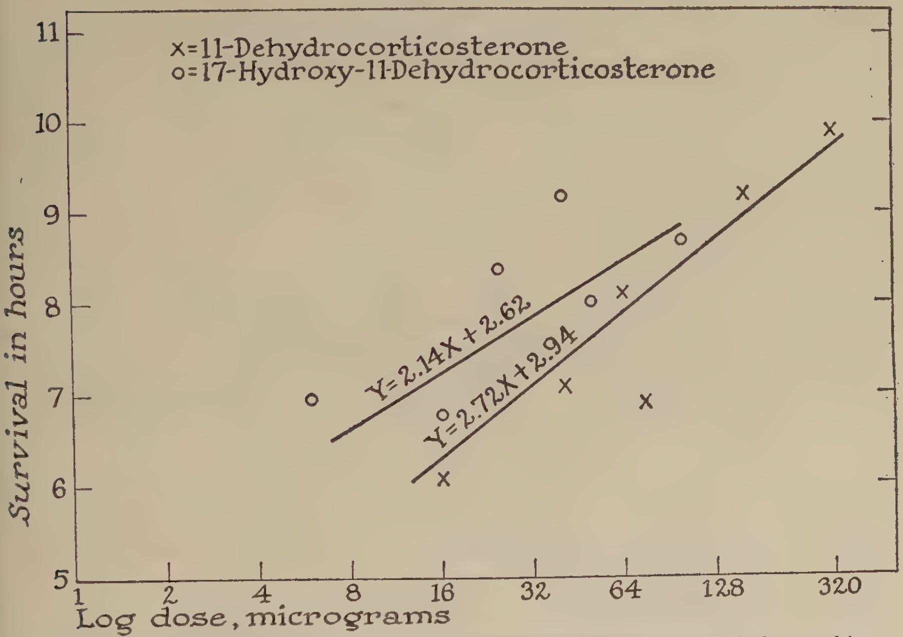


FIGURE 4. Log dose-response curves for 11-dehydrocorticosterone (162 rats) and 17-hydroxy-11-dehydrocorticosterone (113 rats).
Control mean survival was 5.7 hours.

In FIGURES 7 and 8 the comparative responses of an urinary extract to 11-dehydrocorticosterone are illustrated. The relative potencies are tabulated in TABLE 14. A good agreement in the three assays was found, as seen by the fact that, in three assays, 600, 660, and 650 cc. respectively, of urinary extract, were found to be equal in activity to that of one milligram of 11-dehydrocorticosterone.

The determinations of the relative activities of various adrenal cortical steroids are presented in TABLE 15. The expected error of the potency ratio is higher than that for the glycogen methods. 11-Dehydrocorticosterone is about one-third as active as 17-hydroxy-11-dehydrocorticosterone, while corticosterone and 11-desoxycorticosterone acetate are one-twelfth as active by this method.

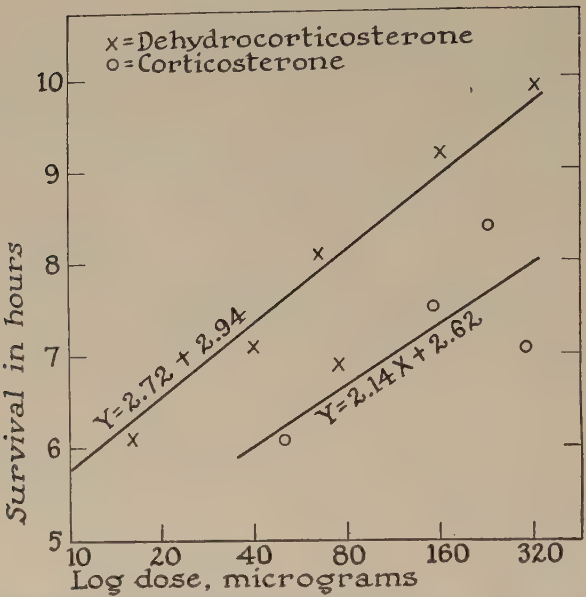


FIGURE 5. Log dose-response curves for 11-dehydrocorticosterone (162 rats) and corticosterone (76 rats). Control mean survival was 5.7 hours.

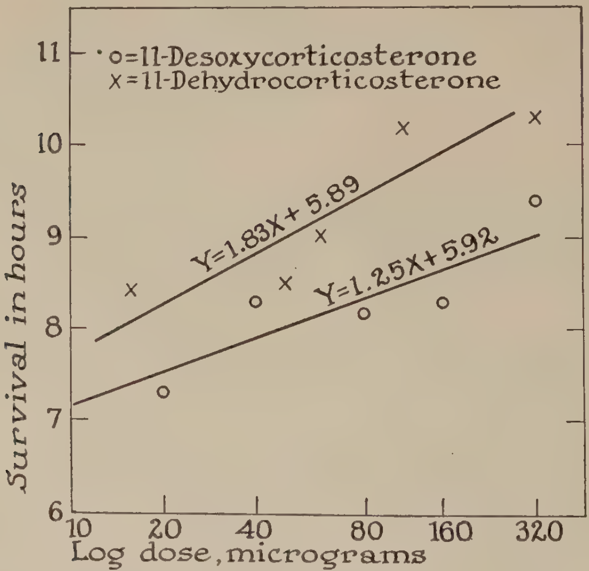


FIGURE 6. Log dose-response curves for 11-dehydrocorticosterone (83 rats) and 11-desoxycorticosterone acetate (52 rats).
Control mean survival was 7.3 hours.

Comparative Activity of Compounds of Various Tests

A summary of the comparative activities of the various adrenal cortical steroids is given in TABLE 16. The activity of 17-hydroxy-11-dehydro-

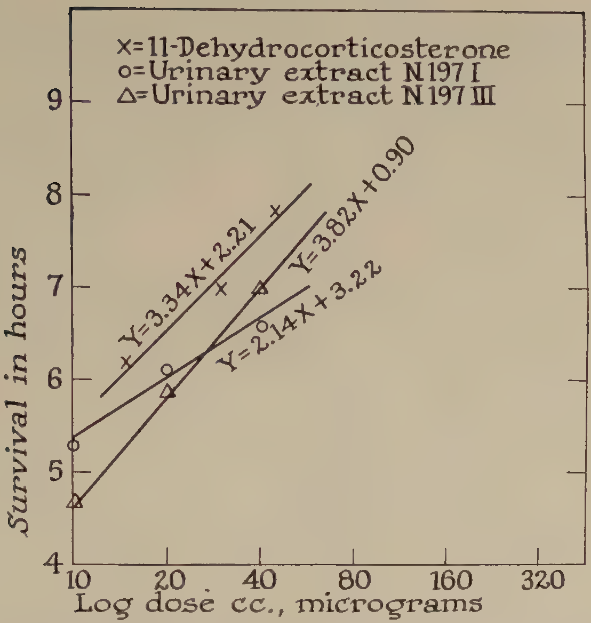


FIGURE 7. The total number of animals used for the curve of 11-dehydrocorticosterone was 51. Each cross represents from 15 to 20 animals. The regression lines were determined by the method of least squares.

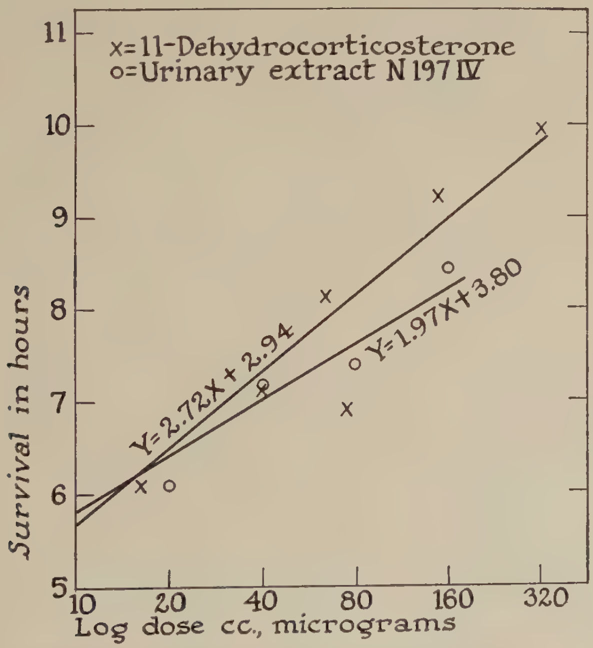


FIGURE 8. The total number of animals used to determine the standard curve was 162. Each cross represents from 7 to 97 animals.

TABLE 14

ASSAY OF POOLED URINARY EXTRACT IN TERMS OF 11-DEHYDROCORTICOSTERONE*

Date	Experiment number	Mg. of 11-Dehydrocorticosterone per liter	Error range ($P = 0.05$)	
			Per cent low	Per cent high
9–14–44	I	0.60	50	102
9–28–44	III	0.66	40	67
1–24–45	IV	0.65	26	35

* From data of Dorfman *et al.*[5]

TABLE 15

THE RELATIVE POTENCIES OF VARIOUS ADRENAL CORTICAL STEROIDS ON THE BASIS OF A COLD PROTECTION TEST*

Compound	Total number of rats	Amount of compound equivalent to one microgram of 11-Dehydrocorticosterone	Error of estimate $P = 0.05$		Remarks
			Low	High	
		μg.	%	%	
17-Hydroxy-11-dehydrocorticosterone	275	0.5	32	46	
	159	0.3	49	93	
	76	0.2	48	92	Compounds run simultaneously
	76	0.3	48	92	Compounds run simultaneously
Corticosterone	238	3.8	35	55	
11-Desoxy-corticosterone acetate	192	4.0	42	71	
	135	4.7	54	115	

* From data of Dorfman *et al.*[4]

TABLE 16

COMPARATIVE ACTIVITIES OF VARIOUS ADRENAL CORTICAL STEROIDS BY VARIOUS METHODS EXPRESSED IN RELATION TO THAT OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE

Compound	Relative potency in terms of 17-Hydroxy-11-dehydrocorticosterone				
	Glycogen rat	Glycogen mouse	Ingle work test	Cold protection test	Growth
17-Hydroxy-11-dehydrocorticosterone	100	100	100	100	100
17-Hydroxycorticosterone	155	—	192	—	219
11-Dehydrocorticosterone	47	28	48	33	—
Corticosterone	54	—	63	9	108
11-Desoxycorticosterone	—	2	2	8	—
17-Hydroxy-11-desoxycorticosterone	—	—	2	—	—

corticosterone has been set at 100, and the values for the other compounds are expressed as percentages of the activity of this hormone. Only those methods are listed where suitable data are available for the estimation.

Comparative Sensitivity and Reproducibility of Various Methods. A summary of the comparative sensitivity and reproducibility of the various methods is presented in TABLE 17. The methods for the assay of materials on the basis of either sodium or potassium metabolism are not well enough established to define the expected errors. In the case of the potassium intoxication test, a rough approximation of ±100 per cent has been listed when 20 animals are used on both the standard and unknown. The amounts needed for this test, as well as sodium excretion in the normal dog, appears to be of a rather high order, 700 micrograms of desoxycorticosterone acetate.

TABLE 17

THE COMPARATIVE SENSITIVITY AND REPRODUCIBILITY OF VARIOUS METHODS FOR THE ASSAY OF ADRENAL CORTICAL MATERIAL

Test	Animal used	Minimum amount of steroid needed per animal		Approximate number of animals on standard	Approximate number of animals on unknown	Error ($P = 0.05$)
		*"E"**	*DCA*†			
		µg.	µg.			%
Sodium excretion	Normal dog	—	700	—	—	—
Potassium intoxication	Adrenal X rat	—	750	20	20	±100
Insulin sensitivity	Normal mouse	500	—	—	—	—
Growth (20 days)	Adrenal X rat	2000	—	26 26	14 29	−32 to +51 −28 to +40
Glycogen	Adrenal X rat	250	—	40 20 10	40 20 10	±15 ±24 ±43
Glycogen	Adrenal X mouse	10	—	40 40	40 10	±20 +50 to +150
Work test	Adrenal X rat	400	—	135	15	±20
Cold test	Adrenal X rat	4	—	100 40	100 40	−35 to +65 −40 to +90

* 17-Hydroxy-11-dehydrocorticosterone.
† 11-Desoxycorticosterone acetate.

The glycogen methods appear to be well established, with the rat method being less variable than the mouse methods, but also less sensitive. The work test, though not particularly sensitive, has a high precision. The cold protecting test is extremely sensitive, but variable enough to decrease its value considerably.

Summary

A survey was made of the methods that have been suggested for the bioassay of adrenal cortical activity from the standpoint of sensitivity and reproducibility. Methods which deal with electrolyte metabolism (sodium and potassium) have not been developed to a high degree of either accuracy or sensitivity.

Methods pertaining to carbohydrate metabolism have been studied in detail and submitted to statistical evaluation. The method of deposition of glycogen in the liver of the fasting adrenalectomized rat is characterized by a high precision, but a relatively low sensitivity. A minimum of 250 micrograms of 17-hydroxy-11-dehydrocorticosterone or its equivalent per animal is required in this test. When a total of 80 animals is employed, half on a standard and half on an unknown, an accuracy of ±15 ($P = 0.05$) can be obtained. The use of a total of 40 animals results in an accuracy of about ±24 ($P = 0.05$), while a total of 20 animals gives an accuracy of about ±43 per cent ($P = 0.05$). The use of the adrenalectomized mouse in place of the rat results in a 25-fold increase in sensitivity, but a decrease in precision.

The work test of Ingle using the nephrectomized-adrenalectomized rat, which is primarily dependent upon the influence on carbohydrate metabolism, has a sensitivity and reproducibility of the order of the rat glycogen method.

The cold protection test, an example of a stress test, is the most sensitive test, but has a lower order of accuracy; and its utility is decreased by the variability in sensitivity from group to group. The growth-survival method of bioassay needs further study for precise evaluation.

The comparative activities of adrenal cortical steroids as determined by the rat glycogen, the mouse glycogen, the work test, the cold test, and a growth method are presented.

References

1. Bülbring, E. 1937. J. Physiol. **89:** 64.
2. Bliss, C. I. 1944. J. Am. Stat. Assoc. **39:** 479.
3. Dorfman, R. I., E. Ross, & R. A. Shipley. 1946. Edocrinology **38:** 178.
4. Dorfman, R. I., R. A. Shipley, E. Ross, S. Schiller, & B. N. Horwitt. 1946. Endocrinology **38:** 189.
5. Dorfman, R. I., R. A. Shipley, S. Schiller, & B. N. Horwitt. 1946. Endocrinology **38:** 165.
6. Eggleston, N. M., B. J. Johnston, & K. Dobriner. 1946. Endocrinology **38:** 197.
7. Feil, M., & R. I. Dorfman. 1945. Endocrinology **37:** 437.
8. Fischer, R. A. 1934. *Statistical Methods for Research Workers.* Oliver and Boyd. London.
9. Gratten, J. F., & H. Jensen. 1940. J. Biol. Chem. **135:** 511.
10. Hartman, F. A., K. A. Brownell, & A. A. Crosby. 1931. Am. J. Physiol. **98:** 674.
11. Ingle, D. J. 1944. *The Chemistry and Physiology of Hormones.* 83. A.A.A.S. Washington.
12. Irwin, J. O. 1937. J. Roy. Stat. Soc. **4:** 1.

13. Olson, R. E., F. A. Jacobs, D. Richert, S. A. Thayer, L. J. Kopp, & N. J. Wade. 1944. Endocrinology **35:** 430.
14. Olson, R. E., S. A. Thayer, & L. J. Kopp. 1944. Endocrinology **35:** 464.
15. Pabst, M. L., R. Sheppard, & M. H. Kuizenga. 1947. Endocrinology **41:** 55.
16. Perla, D., & J. Marmorston-Gottesman. 1931. Proc. Soc. Exp. Biol. & Med. **28:** 1024.
17. Reichstein, T., & C. W. Shoppee. 1943. Vitamins and Hormones. **1:** 346.
18. Reinecke, R. M., & E. C. Kendall. 1942. Endocrinology **31:** 573.
19. Venning, E. H., V. E. Kazmin, & J. C. Bell. 1946. Endocrinology **38:** 79.
20. White, A., & T. F. Dougherty. 1946. Ann. N. Y. Acad. Sci. **46:** 859.

SOME STUDIES ON THE ROLE OF THE ADRENAL CORTEX IN ORGANIC METABOLISM

By Dwight J. Ingle
Research Laboratories, The Upjohn Company, Kalamazoo, Michigan

This paper is concerned with the recent studies in our laboratories on, first, the metabolic effects of adrenal cortical extracts, pure steroids, and pure adrenocorticotrophic hormone, and second, the possible relationship of these effects to some of the metabolic changes which occur during stress.

Background

My interest in this general problem was stimulated by the classical studies of Long and Lukens,[1] who found that pancreatic diabetes in laboratory animals could be alleviated by removal of the adrenal cortices. Large amounts of adrenal cortical extract caused an intensification of the diabetes either in the adrenalectomized-depancreatized animal[2, 3] or in the partially depancreatized, mildly diabetic rat.[3] When pure adrenal steroids were tested for their effects upon carbohydrate metabolism, it was found that 11-desoxycorticosterone was very weak in its diabetogenic and glycogenic activity and that the C-11-oxygenated steroids were much more potent.[4, 5] These studies were further extended by the demonstration of adrenal steroid diabetes in the normal force-fed rat.[6] In rats, the glucosuria always disappears when injections of the steroid are stopped.

The administration of large amounts of cortical extracts or of the C-11-oxygenated adrenal steroids causes an increase in the excretion of non-protein nitrogen, together with an increase in liver glycogen.[4] This is evidence for an increase in gluconeogenesis. It was first believed that the effects of adrenal cortical insufficiency and excess could be completely accounted for in terms of the amount of gluconeogenesis from protein, but further studies showed that the problem was more complex.[7, 5] In studies on the partially depancreatized rat given a constant food intake by stomach tube, Ingle and Thorn[5] found that a mild glycosuria could be abolished by adrenalectomy without any decrease in the amount of urinary nitrogen. Furthermore, the administration of 17-hydroxy-11-dehydrocorticosterone to such rats caused the excretion of much larger amounts of glucose than could be accounted for on the basis of the increase in urinary nitrogen, even if a carbon-to-carbon conversion of protein to carbohydrate was assumed. Such observations led to the conclusion that either the adrenal cortical hormone affects the conversion of fat to carbohydrate or it inhibits the utilization (oxidation, storage or conversion) of carbohydrate. Many investigators are unwilling to accept the assumption that a conversion of fat to carbohydrate occurs to any significant extent. It seems more reasonable to assume that the withdrawal of the cortical hormones in the diabetic animal permits some increase in the utilization of carbohydrate, and that an excess of the cortical hormones causes inhibition of utilization.

To summarize, the amounts of carbohydrate in the blood and tissues of experimental animals are usually depleted in cortical hormone insufficiency and are increased above normal in cortical hormone excess. This correlation is based in part upon the effect of the C-11-oxygenated cortical steroids upon gluconeogenesis from non-carbohydrate sources and perhaps to a greater extent upon the utilization of carbohydrate. In addition, the adrenal cortical hormones may either directly or indirectly influence food intake, absorption, transport, and storage of carbohydrate. This evidence and a more detailed review of the earlier studies on the role of the cortical hormones in organic metabolism have been presented elsewhere.[8]

Current Problems

Some Metabolic Effects of Adrenal Steroids and ACTH

Adrenal Steroid Diabetes in the Normal Rat. How are the effects of the cortical hormones upon the formation and the utilization of carbohydrate related? Drury[9] and Ingle[10] have each suggested that increased gluconeogenesis from protein might represent a compensatory response to a primary inhibitory effect of the cortical hormones on the utilization of carbohydrate. In pancreatic and phlorhizin diabetes and during starvation, there is an increase in protein catabolism which coexists with a decrease in the utilization of carbohydrate. In animals having pancreatic diabetes, control of the glycosuria by treatment with insulin restores a positive nitrogen balance. We[11] have compared the effects of insulin upon nitrogen balance in rats having adrenal steroid diabetes and upon similar animals having pancreatic diabetes. An unanticipated finding was the remarkable insulin resistance of those animals which had adrenal steroid diabetes. This is illustrated in FIGURE 1 by a normal rat which was force-fed a high carbohydrate diet (15 grams of available carbohydrate per day) and made severely diabetic by the administration of 5 mg. daily of 17-hydroxy-11-dehydrocorticosterone. The injection of up to 100 units of protamine zinc insulin per day failed to abolish the glycosuria. In a second animal made diabetic by the administration of 5 mg. daily of 17-hydroxycorticosterone, the administration of up to 1000 units daily of regular insulin failed to control completely the glycosuria. FIGURE 2 gives data on a similar animal in which the hyperglycemia and glycosuria were controlled by protamine zinc insulin without an effect upon the elevated level of urinary non-protein nitrogen. FIGURE 3 shows a typical response of a rat having severe pancreatic diabetes. Treatment with insulin effectively controlled the glycosuria and hyperglycemia and restored a positive nitrogen balance. Although the results of this experiment fail to support the hypothesis under test, they do not fully answer the question. The insulin resistance of the animals having adrenal steroid diabetes prevented our fully controlling the diabetes in all instances.

Which of the adrenal steroids are diabetogenic? In this laboratory we have induced adrenal steroid diabetes in normal rats with corticosterone,[12] 17-hydroxy-corticosterone,[11, 12] and 17-hydroxy-11-dehydrocorticosterone.[11] Massive doses of 11-desoxycorticosterone are diabetogenic in the partially depancreatized rat.[5] The compound 11-dehydrocorticosterone and the "amorphous fraction" of adrenal extracts have not been fully studied.

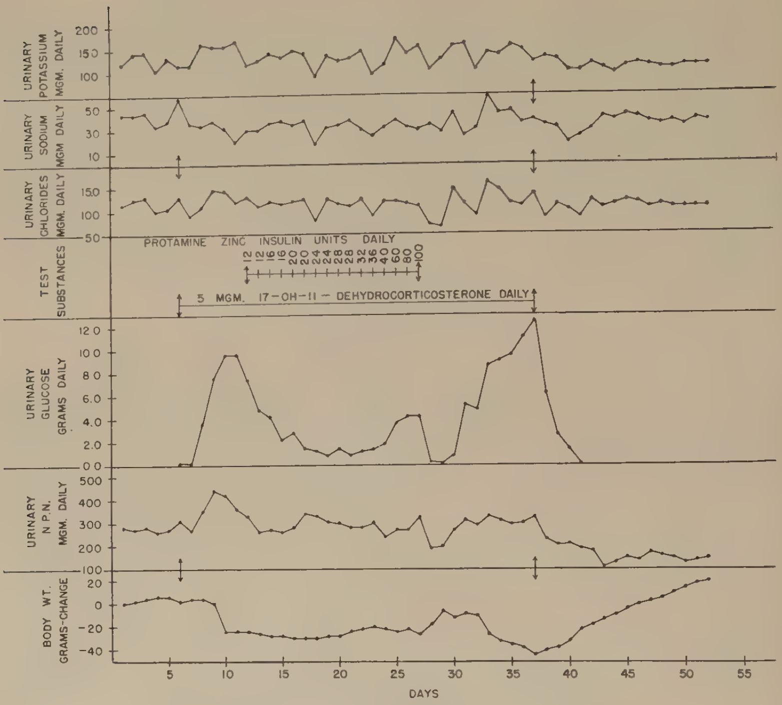


FIGURE 1. Adrenal steroid diabetes in a normal male rat.

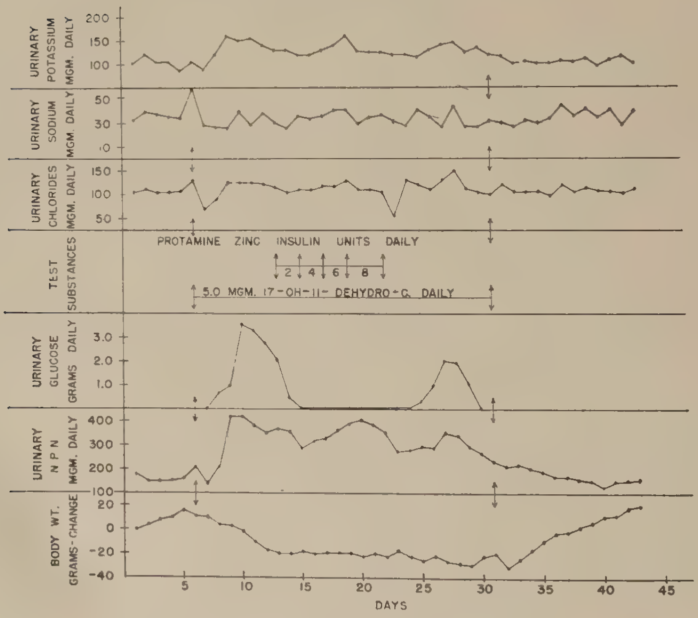


FIGURE 2. Adrenal steroid diabetes in a normal male rat.

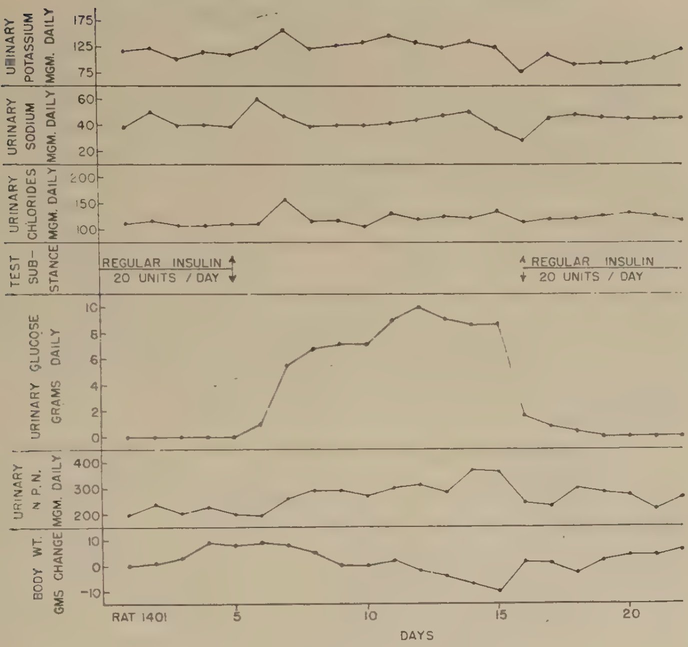


FIGURE 3. Pancreatic diabetes in a male rat.

The Production of Hyperadrenocortinism by Adrenocorticotrophic Hormone in the Rat. Is adrenal steroid diabetes a pharmacologic phenomenon only, or is it within the capacities of the animals' own adrenal cortices to secrete amounts of hormone which will cause similar effects? We have had the privilege of collaborating with Doctors Li and Evans, of the University of California, in studies on the biologic properties of pure adrenocorticotrophic hormone (ACTH). We have reported[13] the production of glycosuria and hyperglycemia in normal rats given ACTH. There was an increase in the excretion of nitrogen and potassium, inhibition of growth, and a manifestation of hyperglycemia and glycosuria just as occurred in similar animals having adrenal steroid diabetes. The results are illustrated in FIGURE 4. It is apparent that the adrenal cortices of rats can secrete the activity equivalent of very large amounts of adrenal cortical extracts or of pure adrenal cortical compounds. Additional evidence on the secretory capacity of the adrenal cortices has been reviewed elsewhere.[14]

The extent to which ACTH will alter the nitrogen balance of the experimental animal may be related to the nature of the diet. Ingle, Prestrud, Li, and Evans (unpublished) compared the effects of ACTH in normal animals force-fed high carbohydrate, high fat, and high protein diets in isocaloric amounts. There was no tendency for ACTH to cause a greater change in nitrogen balance in the rats which received the high protein diet. On the contrary, the administration of ACTH had its smallest effect upon nitrogen balance and body weight in rats force-fed the high protein diet, and its greatest effect upon similar animals maintained on the high fat diet. The

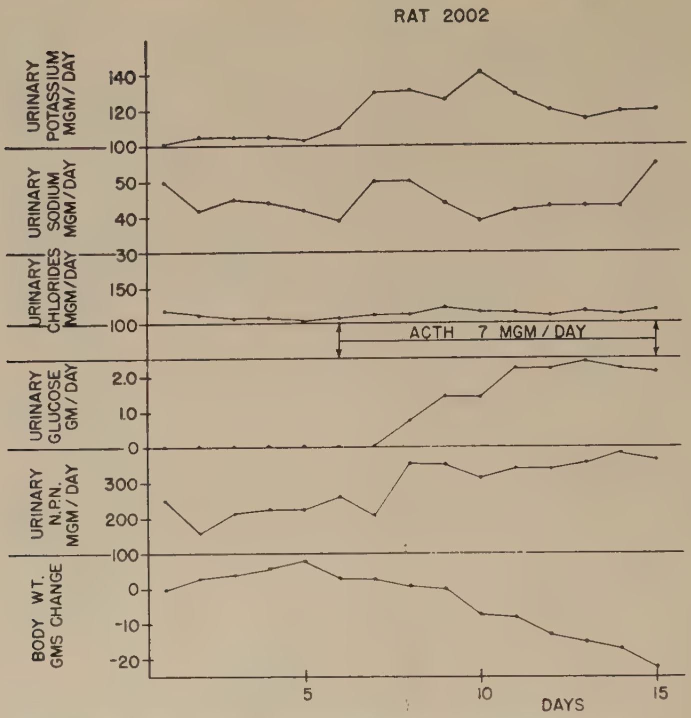


FIGURE 4. Glycosuria caused by adrenocorticotrophic hormone in a normal male rat.

changes in weight paralleled the results on nitrogen balance. The data are summarized in TABLES 1 and 2. Only a small number of animals were tested, but the differences in response were striking and consistent, and are probably valid, thereby giving a basis for adding again to the growing list of interesting questions which relate to the effects of ACTH and the adrenal cortical hormones.

TABLE 1
THE RELATIONSHIP OF DIET TO THE EFFECT OF ACTH UPON URINARY NON-PROTEIN NITROGEN IN THE NORMAL RAT

Daily dose ACTH	Days treatment	*Average daily increase in urinary N.P.N. above control values					
		Increase above values of pre-injection period			Increase above values of control rats		
		High COH	High fat	High protein	High COH	High fat	High protein
mg.							
1	21	54	67	26	44	87	17
3	21	61	77	30	53	88	18
8	10	78	95	64	71	85	42

* Two control and two experimental animals were represented at each level of dosage for each diet, except that 4 rats were given 8 mg. of ACTH per day on the high carbohydrate diet.

TABLE 2

THE RELATIONSHIP OF DIET TO THE EFFECT OF ACTH UPON CHANGE IN WEIGHT IN THE NORMAL RAT. GRAMS CHANGE

Daily dose ACTH	Days treatment	*Average total change in weight during the injection period		
		High COH	High fat	High protein
mg.				
1	21	−10	−17	24
3	21	−28	−33	−8
8	10	−25	−25	−17

* Two control and two experimental animals were represented at each level of dosage for each diet, except that 4 rats were given 8 mg. of ACTH per day on the high carbohydrate diet.

Only the animals on the high carbohydrate diet developed glycosuria during the administration of ACTH in these experiments.

The Effect of Adrenal Cortex Extract Upon the Glucose Tolerance of the Eviscerated Rat. It is frequently assumed that the liver is the sole site of action of the cortical hormones upon carbohydrate metabolism. Is the "diabetogenic" effect of cortical extract mediated by the liver? A number of years ago,[15] cortical extract was found to have a definite effect upon the ability of the eviscerated-nephrectomized-adrenalectomized rat to work when treated with glucose. Russell[16] has shown that the utilization of glucose is increased in the adrenalectomized, functionally eviscerated rat. We have now shown, as is illustrated in FIGURE 5, that the intravenous administration of adrenal cortical extract to the eviscerated rat over a period of 24 hours decreases its tolerance for glucose given by constant infusion.

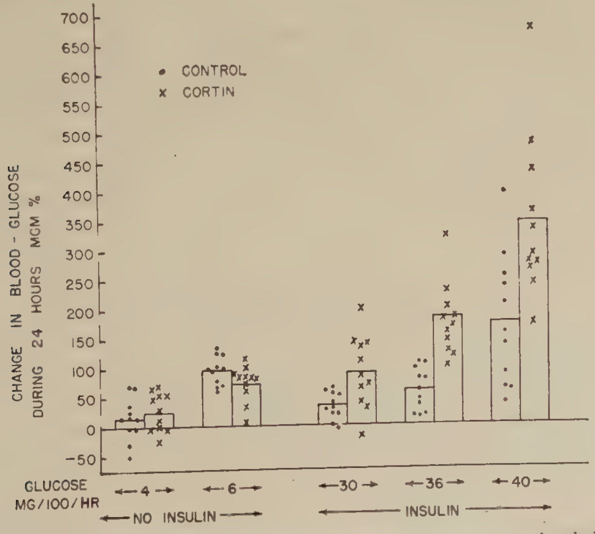


FIGURE 5. The effect of adrenal cortex extract upon the tolerance for intravenously administered glucose in the eviscerated rat. Averages and individual values.

We have observed this effect only in animals which were treated with insulin. The glucose utilization of similar animals without insulin has not been significantly changed by adrenal cortex extract. Is this effect identical with the "diabetogenic" action of the cortical hormones in intact animals? We do not know, but at present there is no reason to assume that it is different. It is a demonstration of an extrahepatic effect, pharmacologic or not, of adrenal cortical extract. How does this effect relate to the action of the cortical hormones in supporting the ability of the eviscerated-adrenalectomized rat to work? It should be interesting to study the effect of the cortical hormones upon glucose tolerance in the eviscerated, working animal.

Adaptation to Adrenal Steroid and ACTH Administration. The early effects of treatment with hormones may not be sustained throughout prolonged administration. This point has been neglected many times. It is an important principle in endocrinology that an appraisal of the physiologic properties of a hormone should be based upon an exploration of the full range of dose-response relationship and of time-response relationship. The limited availability of compounds, such as the adrenal steroids and ACTH, has restricted many studies to 24 or 48 hours and even less. It usually requires a latent period of 72 hours or longer for either the adrenal steroids or ACTH to cause glycosuria. The normal animal is capable of adaptation to the continued administration of a diabetogenic dose of either hormone, so that the glycosuria may disappear. It is not known whether the diabetic state could be sustained indefinitely by a larger dose. The administration of a dose of an adrenal steroid just large enough to induce a negative nitrogen balance in the normal force-fed rat does not sustain the negative nitrogen balance, as is illustrated in FIGURES 6 and 7. The negative nitrogen balance

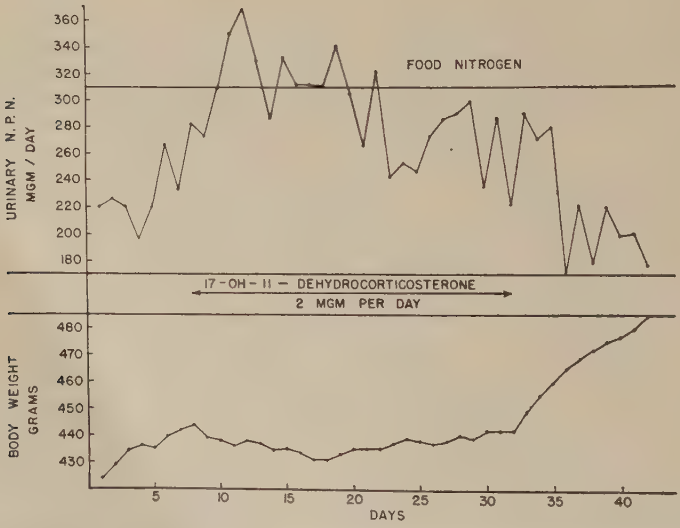


FIGURE 6. Adaptation to the effect of an adrenal steroid upon nitrogen balance in a normal force-fed male rat.

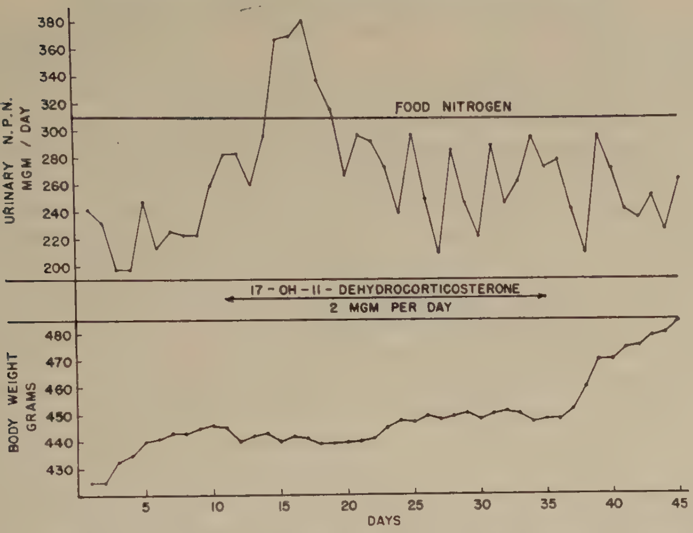


FIGURE 7. Adaptation to the effect of an adrenal steroid upon nitrogen balance in a normal force-fed male rat.

can be re-established by increasing the dose of the hormone. Almost all of the information on the glycogenic effect of adrenal hormones and ACTH has been derived from acute experiments. Can liver glycogen be sustained at a high level by the continued administration of the cortical hormones? Is liver glycogen high in animals having adrenal steroid diabetes, or does it fall to the abnormally low levels which characterize pancreatic diabetes? These and similar questions will require studies of longer duration.

The animal normally lives with its own hormones quietly carrying out their functions with homeostatic mechanisms in equilibrium. An upset of homeostasis by the sudden production of either a hormonal deficiency or an excess may result in non-specific changes which can be confused with those specifically related to the action of the hormone. The acute changes are nonetheless important to observe. In chronic studies of either hormone deficiency or excess, compensating functions of the organism may partially or completely re-establish homeostasis, and thereby mask the effects of the change in hormone intake.

The Possible Role of Hyperadrenocortinism in Some of the Metabolic Adjustments During Stress

The adrenal cortices of laboratory animals are known to hypertrophy and increase their secretory activity during stress. In at least some instances, the accompanying metabolic changes resemble those caused by the administration of either large amounts of the cortical hormones or of ACTH. Are these metabolic changes mediated by an increased secretion of hormones by the adrenal cortices? The following experiments are relative to this problem.

Estrogen Diabetes. Diethylstilbestrol and other estrogens are diabetogenic in the force-fed, partially depancreatized rat.[17] Estrogen diabetes cannot easily be demonstrated in the rat eating *ad libitum*, for the reduction in food intake during the administration of estrogens is so great that the glycosuria may be reduced and the diabetogenic action of the hormone completely masked. Since the administration of diabetogenic amounts of estrogens is damaging to the animal and causes marked adrenal cortical hypertrophy, it seemed reasonable to postulate that the diabetogenic action is due to the increased secretion of diabetogenic adrenal steroids. Our first test of the hypothesis seemed to confirm it, for we were unable to demonstrate a diabetogenic effect of diethylstilbestrol in the adrenalectomized, partially depancreatized rat. The results of the following study[18] make the hypothesis untenable. Partially depancreatized rats, which were without spontaneous glycosuria, became diabetic during the injection of 0.1 mg. of diethylstilbestrol daily, and the glycosuria disappeared when the injections were stopped. The animals were then adrenalectomized and treated with a sub-diabetogenic amount (3 cc. daily) of adrenal cortex extract. Glycosuria developed when diethylstilbestrol was injected and disappeared when the injections were stopped. When the animals were maintained by treatment with either 11-desoxycorticosterone acetate or by drinking 0.9 per cent sodium chloride, the diabetogenic effect of the estrogen was either slight or absent. These data show that the presence of the cortical hormone is essential for a full manifestation of estrogen diabetes, but that an increased secretion of the cortical hormone, in response to the estrogen, is not required.

This study was extended as follows[19]: adrenalectomized, hypophysectomized, partially depancreatized rats were maintained on sub-diabetogenic amounts of adrenal cortical and anterior pituitary extracts. The administration of diethylstilbestrol caused hyperglycemia and glycosuria in all of the test animals which were without glycosuria during the control periods, and, in animals having spontaneous glycosuria, it became more severe when the estrogen was administered.

Diethylstilbestrol also causes an increase of liver glycogen in the intact rat, but not in the untreated adrenalectomized rat.[20, 21] This has been considered proof that the response is mediated by an increase in the secretion of the cortical hormones. A more critical test of the hypothesis in adrenalectomized animals maintained on a steady intake of adrenal cortical extract will be required.

Effects of Other Forms of Stress on Carbohydrate Metabolism

On the supposition that other non-specific damaging agents might be diabetogenic if they caused adrenal cortical hypertrophy and an increased secretion of its hormones, we tested the effect of water intoxication, phenol, formaldehyde, carbon tetrachloride, and cold in the mildly diabetic, partially depancreatized rat.[17, 22] In no case was a diabetogenic effect observed. These studies should be extended by testing other forms of stress over periods of several weeks before the conclusion is drawn that diabetes cannot be intensified by adrenal cortical activation during stress.

When normal fasting rats are subjected to low pressures, there is an increase in the glycogen content of the liver. The response disappears in the adrenalectomized animal,[23, 24] but according to Langley,[25] if the adrenalectomized rat is sustained with adrenal cortical extract in sub-glycogenic doses, anoxia does cause a glycogenic response which is independent of change in the cortical hormone intake. Similarly, crude extracts of the anterior pituitary are diabetogenic in the partially depancreatized rat, but not in the absence of the adrenal glands. Long, Katzin, and Fry[4] have shown that treatment of such animals with adrenal cortical extract maintains the responsiveness to anterior pituitary extract.

The Effect of Adrenalectomy Upon the Excretion of Nitrogen During Forced Feeding and During Fasting

Adrenalectomy in laboratory animals is usually followed by a decrease in the excretion of non-protein nitrogen, thereby indicating a lessened ability of the animal to metabolize protein. Such changes may be due in part to a decrease in intake of food, renal failure, circulatory failure, and other secondary changes of cortical insufficiency. We have studied[26] the nitrogen excretion of adrenalectomized and sham-operated rats during forced feeding and during fasting. The animals were given a 1 per cent solution of sodium chloride to drink during all phases of the experiments. In the first experiment (FIGURE 8), 8 rats were adrenalectomized and 7 were sham-operated.

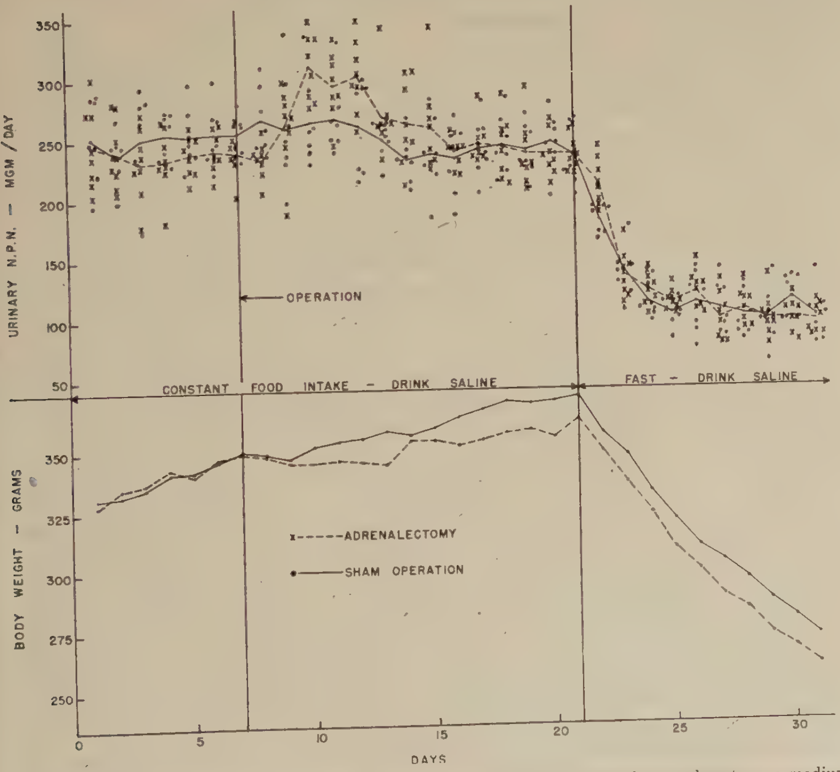


FIGURE 8. Urinary non-protein nitrogen. Averages and individual values from male rats on a medium carbohydrate diet.

During the first postoperative day, the adrenalectomized rats excreted less nitrogen, but by the third day there was a rise in urinary nitrogen which was sustained for several days and which was greater in the adrenalectomized rats than in their controls. This difference in response has been confirmed in five subsequent experiments. During the two weeks of postoperative feeding, the urinary nitrogen of the adrenalectomized group did not decrease significantly below the pre-operative level or that of the control group. During a ten-day fast, the adrenalectomized rats excreted as much urinary nitrogen as their controls. In a second study (FIGURE 9), a medium protein diet was force-fed to 7 pairs of rats. The results paralleled those of the preceding experiment up to the 9th and 10th days of fasting, when the level of urinary nitrogen of the sham-operated rats was definitely higher than that of the adrenalectomized rats.

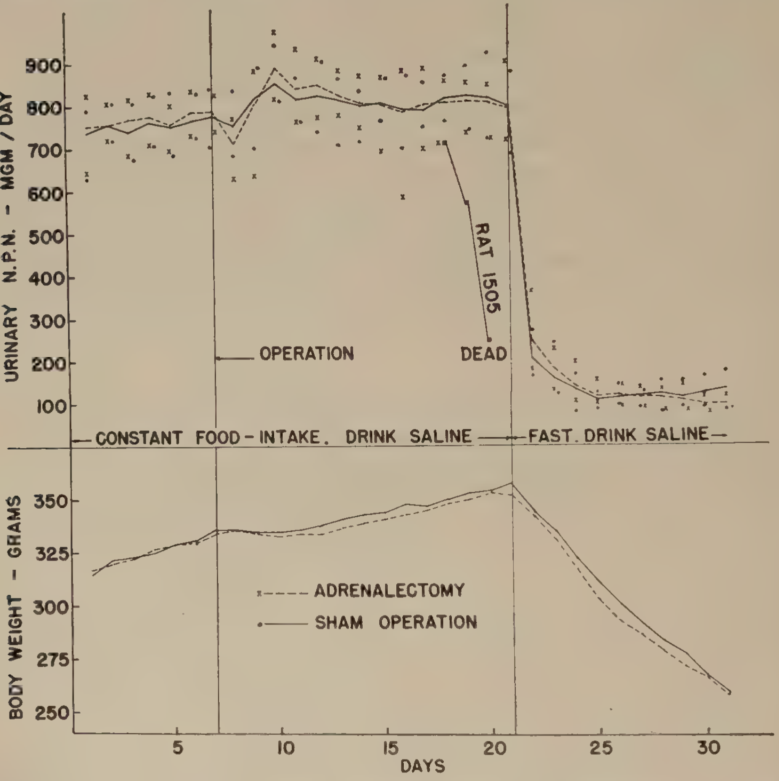


FIGURE 9. Urinary non-protein nitrogen. Averages and range of values from male rats on a medium protein diet.

A similar experiment (unpublished) was carried out with the forced feeding of a high protein diet. The average amount of nitrogen excreted by the adrenalectomized, saline-treated animals was significantly less than was excreted by their sham-operated controls; but there was overlapping in the individual values, and amounts of non-protein nitrogen up to 1530 mgm. per day were excreted by some of the adrenalectomized group. Data on the two longest surviving animals are shown in FIGURE 10. Symptoms of cortical insufficiency were manifest within the first few days, and all of this group died within 14 days following adrenalectomy. There was failure of absorption and an eventual development of diarrhea in all of the adrenalectomized animals. In contrast, non-adrenalectomized rats can withstand considerable overfeeding of this diet, and we have observed daily values for urinary non-protein nitrogen up to 2100 mgm. before the animals were killed by overfeeding.

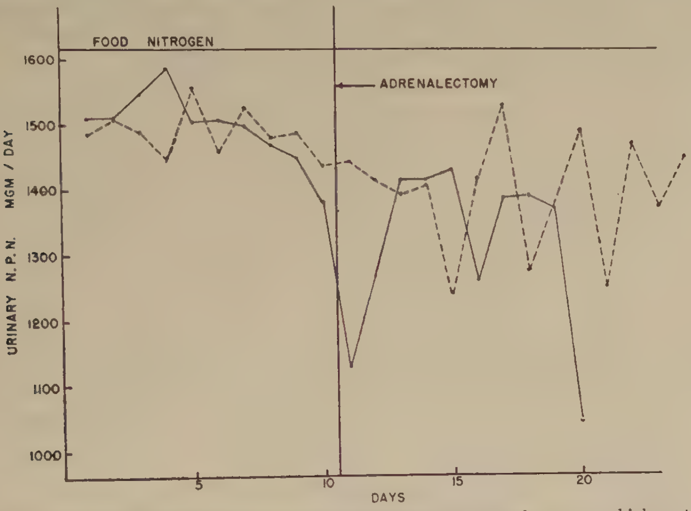


FIGURE 10. The effect of adrenalectomy upon urinary nitrogen in two male rats on a high protein diet .

These and other experiments[27] show that it is possible for adrenalectomized rats to metabolize large amounts of fed protein when maintained in good condition on a favorable intake of sodium chloride. None of our force-fed animals showed any degree of failure until they became sick. The failure of the animal to metabolize fed protein may be secondary to a failure of supporting mechanisms, particularly circulatory mechanisms. However, the ease with which one may make this assumption should not be mistaken for proof. Nothing has been shown of the kinetics of protein catabolism or of the turnover of amino acids between the body fluids and tissues, and it has not been shown that the adrenalectomized, saline-treated rat can tolerate as great a dietary load as the non-adrenalectomized rat. The question is unsettled.

Is the adrenalectomized animal able to mobilize its body proteins as well as does the intact animal? Although a number of studies[4] have shown that it does not, there was little evidence that our adrenalectomized rats differed from normal in their excretion of non-protein nitrogen during fasting. Our studies differed from earlier acute experiments in that adrenalectomized rats were maintained for two weeks by forced-feeding diets relatively low in carbohydrate prior to fasting. The fasting rat has a very low level of nitrogen excretion as compared to other animals, but it can mobilize its own tissues very rapidly during stress. In order to test fully the ability of the adrenally insufficient animal to mobilize its own tissues, the comparison should be made under conditions where the rate of mobilization is stimulated in the intact animal. Some such studies are to be described.

The Effect of Fractures on Nitrogen Excretion in Adrenalectomized and Sham-Operated Rats

In the first experiment (FIGURE 11), 12 rats were maintained on the medium carbohydrate diet for two weeks, at which time 6 rats were adrenalectomized and 6 were sham-operated. All of the rats were given 0.9 per cent sodium chloride to drink during the entire experiment. No hormone was given in this experiment. Two weeks following operation all of the animals were subjected to fracture of the knee-joint and tibia of the right hind leg. There was a rise in nitrogen excretion following operation in each group, again higher in the adrenalectomized rats; but subsequently there was no significant rise following fractures in the adrenalectomized animals such as occurred in the non-adrenalectomized rat.

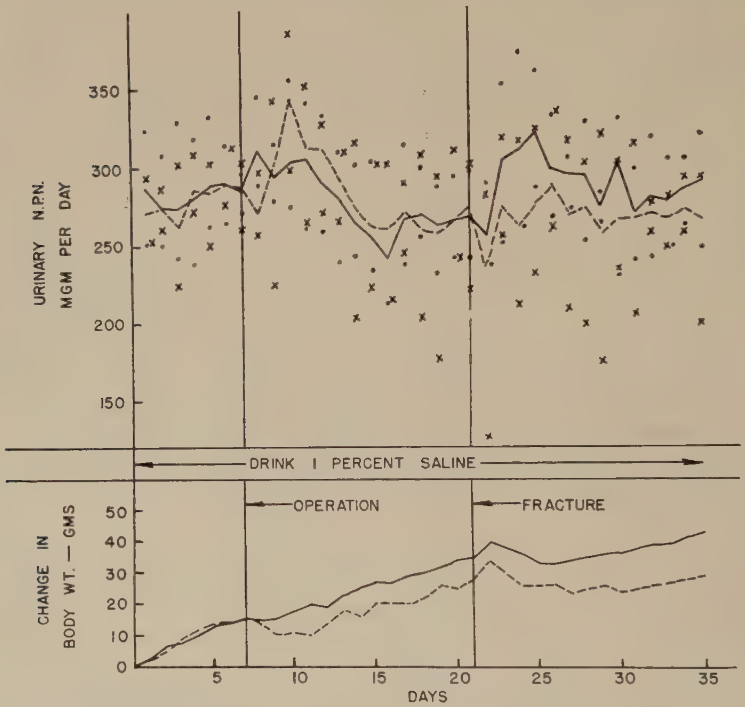


FIGURE 11. The effect of fractures upon urinary N.P.N. in adrenalectomized and sham-operated male rats. Averages and range of values.

Recalling the results of our experiments[18] on the diabetogenic activity of diethylstilbestrol in adrenalectomized rats, the next step was to substitute a constant intake of adrenal cortex extract for the adrenal glands and to test again the response to fractures. Accordingly, in the second experiment (FIGURE 12), treatment with saline was omitted and the adrenalectomized rats were treated with 4 cc. daily of beef adrenal extract, from the time of operation throughout the following four weeks. The rise in nitrogen excretion following operation was less in the cortin-treated animals than in their sham-operated controls and less than was shown by the saline-treated, adrenalectomized animals of the preceding experiment (FIGURE 9). Additional study will be required to determine whether there is a true difference in the response of saline-treated and cortin-treated rats in respect to nitrogen loss following adrenalectomy. Following the fracturing of the tibia and knee-joint, the adrenalectomized, cortin-treated rats excreted less nitrogen during the first 24 hours, but thereafter showed a rise in nitrogen excretion which was even greater than that of the control series.

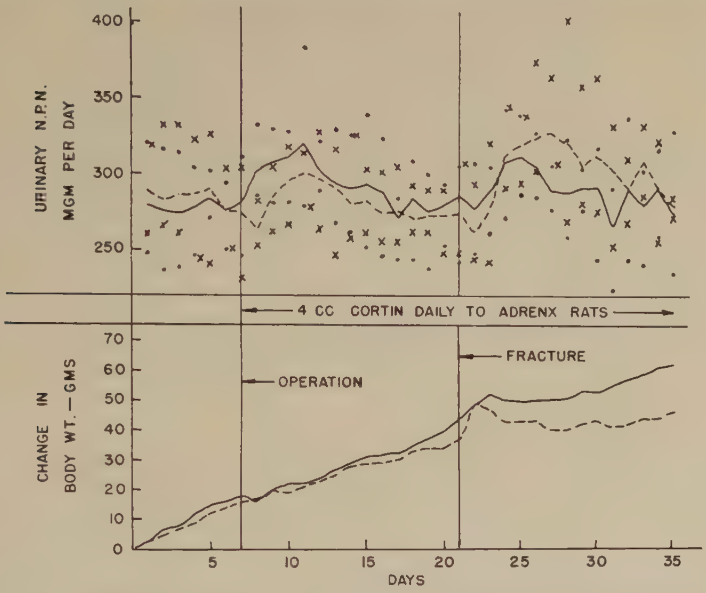


FIGURE 12. The effect of fractures upon urinary N.P.N. in adrenalectomized rats treated with cortica extract and sham-operated male rats. Averages and range of values.

In the third experiment (FIGURE 13), the conditions were identical with those of the preceding study, except that the tibia, femur, and knee-joint of both hind legs were fractured in each rat. The results were similar to those of the preceding study, except that the post-fracture rise in nitrogen excretion was greater in both the adrenalectomized and non-adrenalectomized animals.

Here is an example of the subnormal ability of the untreated adrenalectomized animal to mobilize its tissue proteins following damage. Does this reflect a primary deficiency of some prepotent function of the cortical hormones? The adrenalectomized animals of this study appeared to be

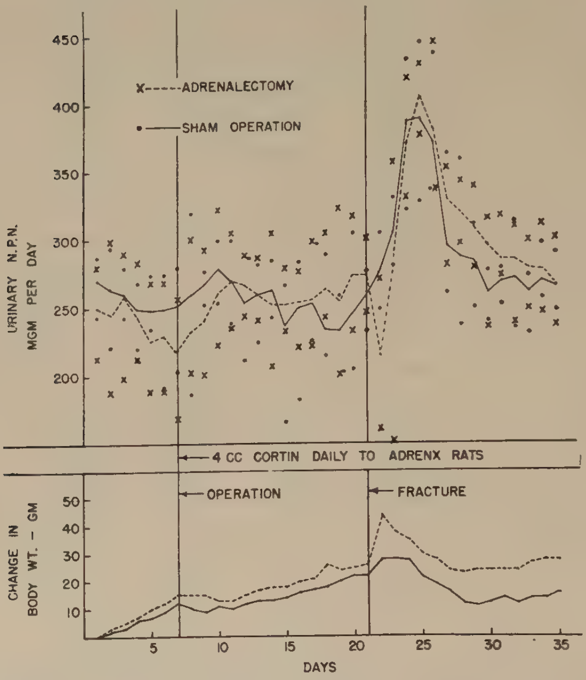


Figure 13. The effect of extensive fractures upon urinary N.P.N. in adrenalectomized rats treated with adrenal cortex extract and sham-operated male rats. Averages and range of values.

mildly "sick" following the fractures. Did we deal with a primary or a secondary deficiency? We do not know. The hypothesis, that the post-fracture rise in nitrogen is due specifically and solely to an increased secretion of the cortical hormones following stress, is clearly incorrect. The presence of the hormones was required to maintain the responsiveness of the tissues, but an increase in hormone was not the only cause of the response.

In all of our experiments, there has been a difference in the nitrogen excretion of non-adrenalectomized rats and either cortin- or saline-treated, adrenalectomized rats during the first 24 hours following an operation or a fracture. This either may reflect a difference in the dissolution of lymphoid tissue,[28] which does seem to occur rapidly and extensively only in response to increased adrenal cortical activity, or it may reflect secondary factors.

The Effect of Burns on Nitrogen Excretion in Adrenalectomized and Cortin-Treated Rats. A rise in the excretion of urinary nitrogen is a typical response to an extensive burn. As shown in FIGURE 14, this response occurs in adrenalectomized, cortin-treated rats just as in the sham-operated controls and was therefore independent of any change in adrenal activity. Among four adrenalectomized rats treated with saline, only two animals showed a marked negative nitrogen balance following a burn, and two failed to show a significant increase in urinary nitrogen, but did manifest diarrhea and weakness.

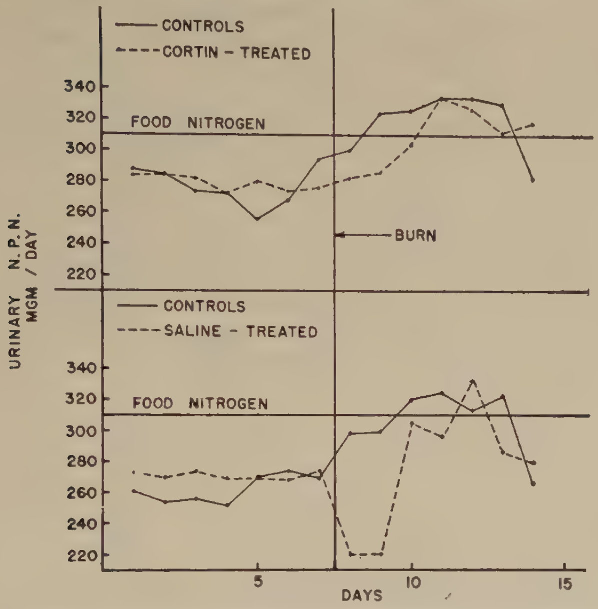


Figure 14. The effect of burns upon urinary N.P.N. in adrenalectomized rats, with and without cortical hormone, and in control rats. Averages.

The Effect of Thyroxin Upon Nitrogen Balance in Adrenalectomized Rats. It can be argued that the nitrogen lost following an extensive fracture or a burn may arise from the autolysis of damaged tissue and that is not mobilized by physiological mechanisms. This may or may not explain all of the loss of tissue protein following tissue injury; nevertheless, the animal is able to catabolize it to nonprotein compounds, for the nitrogen is excreted as such. In an effort to test the ability of the adrenalectomized rat to catabolize its own tissues by means of non-pathological mechanisms, thyroxin was administered to eight adrenalectomized, saline-treated rats. Two of these animals showed a striking response, as is illustrated by the data on one rat in figure 15. Although all of them showed an increase in nitrogen loss, some developed symptoms of adrenal cortical insufficiency and died without showing a normal response. The effect of thyroxin in the cortin-treated, adrenalectomized animal has not been studied by us. A number of years ago, a highly important study of the effect of thyroxin in the partially adrenalectomized dog was made by Koelsche and Kendall.[29] In brief, it was shown that the partially adrenalectomized dog was more easily thrown into negative nitrogen balance by the administration of thyroxin than the normal dog and that treatment of the partially adrenalectomized dog with cortical extract raised the resistance of the animal to thyroxin and restored a positive nitrogen balance.

Discussion

It is abundantly evident from our own and other studies that the adrenal cortex is not the sole regulator of protein breakdown and that other adaptive

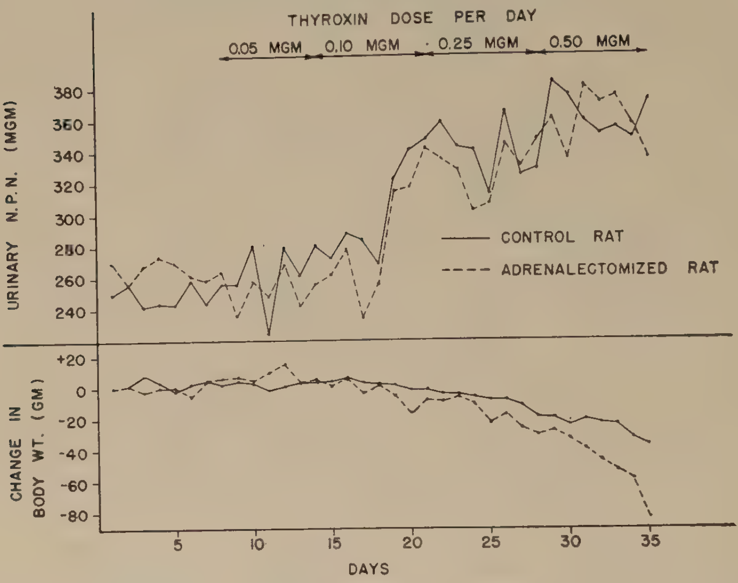


FIGURE 15. The effect of thyroxin upon urinary N.P.N. in one adrenalectomized and one control rat.

mechanisms can operate in the absence of any change in the intake of the cortical hormones. This is in keeping with established principles of physiology. In the entire field of physiology, I do not know of any metabolic process which is exclusively the function of any single organ. On the other hand, the cortical hormones can indeed affect the metabolism of protein, for there are the well-established effects of the C-11-oxygenated steroids in causing the breakdown of lymphoid and other tissues and in inhibiting growth. Albright[30] has offered the attractive hypothesis that the effect of the cortical hormones upon the structural proteins and growth may represent a blocking of protein synthesis, an "anti-anabolic" rather than "catabolic" effect. The breakdown of lymphoid tissue is too rapid to be explained on such a basis and must reflect, at least in part, a "catabolic" action. The nature of the effect on structural tissues is less certain.

The production of hyperadrenocortinism by the administration of either the cortical hormones or ACTH is characterized by a diabetogenic effect in the rat and a negative nitrogen balance. Why are not these same metabolic changes caused by adrenal cortical hyperfunction when it is stimulated by stress? It has been suggested[18] that the increased secretion of the cortical hormones to meet an increased need for their function does not cause overdosage phenomena but serves only to maintain homeostasis. It is certain, at least, that the response of the adrenal cortex during stress serves some purpose which is essential for normal resistance.

The data of these studies emphasize the inhibitory effect of the cortical hormones upon carbohydrate utilization rather than its positive effect upon gluconeogenesis. But how does inhibition of carbohydrate utilization relate to the effect of these hormones upon body economy? Of what use is a "diabetogenic" action? Adrenal steroid diabetes is an overdosage phenomenon, but it may represent an exaggeration of normal function. Possibly, it favors the oxidation of fat and conserves carbohydrate for emergency use during a stress or during fasting.

The brilliant studies of Stetten[31, 32] indicate that the conversion of carbohydrate to fat is a normal and important pathway in its utilization and that one of the principal defects in diabetes is the inability of the animal to convert carbohydrate to fat. In studies on the overfeeding of a high carbohydrate diet, we[33] have observed that normal rats can tolerate enormous amounts of fed carbohydrate by storing it as fat. Why cannot animals having either pancreatic or adrenal steroid diabetes dispose of the excess blood glucose by converting it to fat? They do not conserve glucose in either case, but waste it by excretion into the urine. In adrenal steroid diabetes, there may also be an inability of the animal to convert its carbohydrate to fat. To the contrary, there is some evidence that the cortical hormones favor the storage of fat.[34, 35]

How are the various effects of the cortical hormones upon organic metabolism related one to the other? In this paper, I have described an unsatisfactory attempt to explain the stimulation of gluconeogenesis as a compensatory response to the inhibitory action upon the utilization of carbohydrate. The studies of Price *et al.*[36] have shown that certain adrenal cortical principles modify the hexokinase reaction by intensifying the inhibitory effect of anterior pituitary extract upon the action of insulin. Stetten[37] has suggested that the position of the hexokinase reaction among the processes of carbohydrate utilization would permit a possible explanation of the effects of the adrenal cortical hormones upon the formation, storage, conversion, and oxidation of carbohydrate. There are at least two arguments against this hypothesis: First, the adrenal hormones exert a diabetogenic and glycogenic action in the hypophysectomized animal, whereas, in the studies of Price *et al.*,[36] the cortical hormones did not affect the hexokinase reaction in the absence of anterior pituitary extract. Second, Price *et al.* obtained marked effects with the amorphous fraction of cortical extracts and little or no effect with the compounds 11-dehydrocorticosterone, corticosterone, and 17-hydroxy-11-dehydrocorticosterone which are active in the intact animal. It is possible to explain these discrepancies by assuming that the hypophysectomized rat has a trace of either anterior pituitary hormone or anterior pituitary-like hormone which synergizes the action of the cortical hormone and, secondly, that the C-11-oxygenated steroids failed to show activity in the *in vitro* experiments because of their insolubility.

The thinking of many of us in the adrenal field has become too rigidly channelized during the past few years. It is common to describe adrenal physiology in terms of electrolyte and organic (generally limited to protein and carbohydrate) metabolism as though they are completely unrelated functions, electrolyte metabolism being controlled by 11-desoxycorticosterone and organic metabolism being controlled by the C-11-oxygenated steroids. The story is not this simple. It is true that 11-desoxycorticosterone has its most striking effect upon electrolyte balance and that only the C-11-oxygenated compounds are potent in their effects upon carbohydrate metabolism. But there is considerable overlapping of effects, and there is a great deal of evidence that there are basic relationships between the distribution of inorganic ions and the metabolism of organic compounds which relate to adrenal cortical functions.

We in the adrenal field have been observing the end results of cortical hormone deficiency and cortical hormone action, and have spoken of end results as though they were the processes of action. We know none of the reactions into which the cortical hormones enter to bring about their metabolic effects. The metabolic changes which result from cortical insufficiency and excess are nonetheless important to consider. The correction of sodium loss and carbohydrate deficiency in cortical insufficiency may determine whether the animal or patient is able to live. But, not all of the effects of the cortical hormones upon the viability and vigor of the animal can be defined in terms of what is now known of the metabolic effects of these hormones. In the non-adrenalectomized animal, the sodium and chloride content of the blood can be depleted to levels lower than occur in adrenal cortical insufficiency without causing death. Similarly, lower values for blood glucose and tissue glycogen can be tolerated by non-adrenalectomized animals. Non-adrenalectomized animals can tolerate higher levels of serum potassium than usually occur in cortical insufficiency. Many of the symptoms of cortical insufficiency resemble those of animals dying of other causes. Other steroid compounds, particularly estrogens, have a corticomimetic action in that they cause sodium retention and exert a diabetogenic action without any favorable effect upon the survival and vigor of the adrenalectomized animal.

The most important and remarkable advances in the adrenal field have been the isolation, identification, and synthesis of several biologically active compounds. The practical advances in the treatment of adrenal disease and many of the advances in physiology awaited the contributions of the chemist. Now the new techniques of enzymology and isotope chemistry may be the tools required to probe into the processes by which the cortical hormones produce their effects. In addition to techniques, new and broader concepts are needed. I hope that there will remain a place for physiologists who can advance the methods of experimenting upon animals and who will, at appropriate intervals, remind the chemist that the organism functions as a whole.

Bibliography

1. Long, C. N. H., & F. D.W . Lukens. 1936. J. Exp. Med. **63:** 465.
2. Lukens, F. D. W., & F. C. Dohan. 1938. Endocrinology **22:** 51.
3. Long, C. N. H. 1939. Trans. & Stud. Coll. Phys. Philadelphia **7:** 21.
4. Long, C. N. H., B. Katzin & E. G. Fry. 1940. Endocrinology **26:** 309.
5. Ingle, D. J., & G. W. Thorn. 1941. Am. J. Physiol. **132:** 670.
6. Ingle, D. J. 1941. Endocrinology **29:** 649.
7. Thorn, G. W., G. F. Koepf, R. A. Lewis, & E. Olsen. 1940. J. Clin. Invest. **19:** 813.
8. Ingle, D. J. 1945. *The Chemistry and Physiology of Hormones:* 83–103. A. A. A. S. Washington, D. C.

9. Drury, D. R. 1942. Endocrinology **2:** 421.
10. Ingle, D. J. 1942. Endocrinology **31:** 419.
11. Ingle, D. J., R. Sheppard, J. S. Evans, & M. H. Kuizenga. 1945. Endocrinology **37:** 341.
12. Ingle, D. J., R. Sheppard, E. A. Oberle, & M. H. Kuizenga. 1946. Endocrinology **39:** 52.
13. Ingle, D. J., C. H. Li, & H. M. Evans. 1946. Endocrinology **39:** 32.
14. Ingle, D. J. 1944. Endocrinology **4:** 208.
15. Ingle, D. J. 1938. Proc. Soc. Exp. Biol. & Med. **39:** 151.
16. Russell, J. A. 1943. Am. J. Physiol. **140:** 98.
17. Ingle, D. J. 1941. Endocrinology **29:** 838.
18. Ingle, D. J. 1943. Am. J. Physiol. **138:** 577.
19. Ingle, D. J. 1944. Endocrinology **34:** 361.
20. Long, C. N. H. 1942. Endocrinology **30**: 870.
21. Janes, R. G., & W. O. Nelson. 1942. Am. J. Physiol. **137:** 557.
22. Ingle, D. J., & J. Nezamis. 1943. Endocrinology **33:** 181.
23. Evans, G. 1936. Am. J. Physiol. **114:** 297.
24. Lewis, R. A., G. W. Thorn, G. F. Koepf, & S. S. Dorrance. 1942. J. Clin. Invest. **21:** 33.
25. Langley, L. L., & R. W. Clarke. 1942. Yale J. Biol. & Med. **14:** 529.
26. Ingle, D. J., & E. A. Oberle. 1946. Am. J. Physiol. **147:** 222.
27. Wells, B. B., & E. C. Kendall. 1940. Proc. Staff Meet., Mayo Clinic **15:** 565.
28. Dougherty, T. F., & A. White. 1944. Endocrinology **35:** 1.
29. Koelsche, G. A., & E. C. Kendall. 1935. Am. J. Physiol. **113:** 335.
30. Albright, F. 1943. *The Harvey Lectures.* Science Press. Lancaster, Pa.
31. Stetten, D., Jr., & B. V. Klein. 1945. J. Biol. Chem. **159:** 593.
32. Stetten, D., Jr., & B. V. Klein. 1946. J. Biol. Chem. **162:** 377.
33. Ingle, D. J. 1946. Endocrinology **39:** 43.
34. Kochakian, C. D. 1944. Josiah Macy, Jr. Foundation, Conference on Metabolic Aspects of Convalescence. Transactions of 6th Meeting. 13.
35. Kendall, E. C. 1945. Josiah Macy, Jr. Foundation, Conference on Metabolic Aspects of Convalescence. Transactions of 10th Meeting. 81.
36. Price, W. H., M. W. Slein, S. P. Colowick, & G. T. Cori. 1946. Federation Proceedings **5:** 150.
37. Stetten, D., Jr. 1946. J. A. M. A. **132:** 373.

THE CYTOLOGY AND CYTOCHEMISTRY OF THE ADRENAL CORTEX*

By Roy O. Greep and Helen Wendler Deane
Harvard School of Dental Medicine and Department of Anatomy, Harvard Medical School, Boston, Massachusetts

The present paper concerns itself with the cytological and cytochemical aspects of the adrenal cortex. We have endeavored to interpret whatever morphological and chemical changes have been detected in terms of the demand for and the evidence of hormone production. The adrenal cortex is exceptionally well adapted for cytophysiological and cytochemical study because clear-cut alterations in its chemical and structural make-up follow quickly any disturbance in the bodily equilibria with which the cortex is concerned. Moreover, considering that high local concentrations of compounds are an important requisite to a cytochemical study, the cortical constituents which fulfill this requirement are triglycerides, steroids and ascorbic acid.

Histology of the Adrenal Cortex

The functional elements, or parenchyma, of the cortex are large polygonal epithelial cells. These cells comprise the great bulk of the tissue. On the basis of differences in the arrangement of the parenchymal cells, Arnold divided the cortex into concentric layers which, from the periphery inward, are named: the *zona glomerulosa*, made up of loops and balls of cells; the *zona fasciculata*, having long columns of cells; and the *zona reticularis*, in which the fascicular columns become distorted and broken.[2] The fasciculata has been subdivided further on the basis of the lipid droplets contained within its cells into a thin subglomerular region referred to variously as the transitional, lipid-free, or "sudanophobic"[60] zone, a broad and very fatty outer fasciculata containing the so-called spongiocytes, and an equally broad inner zone in which the lipid droplets of the cells are smaller and less numerous.

The cortex has an extremely rich vascular bed.[3] Arterioles enter from the capsule and immediately break up into numerous long sinusoids, which run radially between the columns of cells (FIGURE 1). The cells near the capsule are therefore more advantageously situated with respect to a fresh supply of oxygenated blood. Parenthetically, it may be remarked that the adrenal cortex appears to lack innervation and is, therefore, probably entirely under hormonal control. The connective tissue stroma consists of a reticulum of argyrophil fibers which underlies the sinusoids and enmeshes the cells.

Cytology of the Adrenal Cortex

Under the present heading, we shall consider the mitochondria, or chondriosomes, the Golgi apparatus, and the lipid droplets. The first two of

---

* The observations reported here were supported, in part, by a grant to the Department of Anatomy from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.

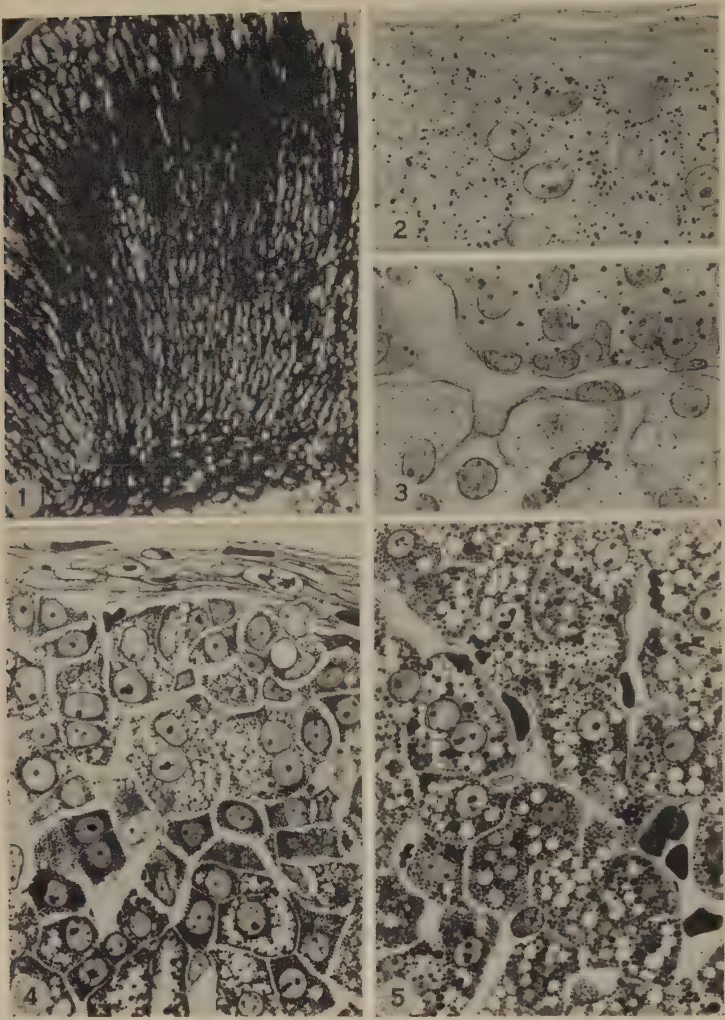
these structures are the subject of an extensive literature for which comprehensive reviews are available,[2, 51, 63] so that there appears to be no point in discussing them here, beyond examining their usefulness for characterizing the various zones of the cortex and noting whatever changes may occur in different states of activity. A valuable recent review concerning the general nature and activity of the mitochondria and Golgi apparatus is to be found in Bourne's *Cytology and Cell Physiology.*[8]

Mitochondria

With improved techniques, the tendency in recent years has been to demonstrate fewer and fewer bizarre forms of chondriosomes in the cortical cells; the rings, beads, clubs, etc. have largely disappeared from descriptions. At present, the mitochondria of the cortex of normal animals are observed to be either spherical granules or short rods, depending on the species. Pertinent studies are available for the guinea pig,[35] cat,[2] and rat.[16]

The cells of the glomerulosa have an exceedingly dense population of mitochondria, which in the guinea pig and cat are rod shaped, but which in the rat are small, uniform spherules. In the guinea pig and cat, they are distributed quite evenly throughout the cytoplasm, thus giving these cells, which have relatively few fat vacuoles, a homogeneous appearance. In the rat, where the glomerulosa contains more numerous lipid droplets, they lie in the interstices between the droplets (FIGURE 4). In the cells of the fasciculata, the mitochondria are spherical in all the species which have been studied, and occur in the protoplasmic films between the lipid droplets. In the rat, the only morphological difference which we have noted between these two major zones is that the mitochondrial spherules are larger in the fasciculata than in the glomerulosa (FIGURE 5). The reticularis is characterized mainly by an extraordinary variability in the size and number of the chondriosomes. In some cells they are large, in others small, but within any given cell the size is generally regular. In this zone, occasional "light" cells have only a few mitochondria and contrast sharply with "dark" cells in which the mitochondria are so densely packed that it is difficult to recognize other structures in the cytoplasm. This difficulty is enhanced by the fact that the cytoplasm of the "dark" cells stains intensely with iron-alum hematoxylin. Because of this property, the "dark" cells are frequently called siderophiles. Furthermore, in some cells of the reticularis the mitochondria are fragmented, stain poorly, and are distributed irregularly.[2] These phenomena are ordinarily associated with cell degeneration.

We have examined the mitochondria in the various zones of the rat adrenal following hypophysectomy,[16] and Deane and co-workers[17, 19] have made similar studies on rats in which the adrenals had been stimulated to hyperactivity. Hypophysectomy does not alter the mitochondria in the glomerular zone, but in the spongy or outer layer of the fasciculata their size is markedly reduced, so that they come to resemble those of the glomerulosa.[16] In rats subjected to deficiencies of pantothenic acid[17] or of thiamine,[19] in both of which there is evidence of hyperactivity of the zona fasciculata, the mitochondria of this zone are enlarged and become less chromophilic. When



FIGURES 1–5

FIGURE 1. Section of a cat's adrenal which had been injected with dilute India ink via both the aorta and the vena cava so as to visualize the sinusoids. The sinusoids can be seen outlining the glomerulosal arcades and then paralleling the cell columns of the zona fasciculata toward the medulla. The reticularis is visible in the lower part of the figure. The sinusoids are particularly narrow in the outer portion of the fasciculata. From BENNETT & KILHAM.[3] × 58.

(FIGURES 2–5 are drawings with the aid of a camera lucida, made by Etta Piotti.)

FIGURE 2. Rat's adrenal cortex treated with acid silver nitrate to demonstrate ascorbic acid. Capsule and zona glomerulosa. Small granules of reduced silver are present in the cytoplasm of the glomerulosal cells and also in the inter-cellular spaces (probably the sinusoids). DEANE & MORSE.[18] × 1180.

FIGURE 3. Ascorbic acid in the zona reticularis (above) and the medulla of rat's adrenal, prepared as FIGURE 2. Large granules in the cytoplasm of the cells of the reticularis. Fine precipitate in the medullary cells; a macrophage shows an especial concentration of ascorbic acid. DEANE & MORSE.[18] × 1180.

FIGURE 4. Mitochondrial preparation of rat's adrenal cortex. Fixed in Zenker-formalin, stained with iron-alum hematoxylin. Shows capsule, zona glomerulosa, transitional zone (containing no lipid spaces), and a few cells of the outer fasciculata. The mitochondria in the cells of the glomerulosa are small spheres, those of the fasciculata somewhat larger spheres. DEANE & GREEP.[16] × 1180.

FIGURE 5. Mitochondrial preparation of rat's adrenal cortex, showing the middle part of the fasciculata. The cells contain spherical mitochondria (gray), large liposomes (black), and numerous lipid spaces. The mitochondria are somewhat larger than those of the glomerulosa. DEANE & GREEP.[16] × 1180.

the dietary deficiencies exceeded toleration, the mitochondria become vesiculated.

These experimental studies suggest that the mitochondrial form is a useful measure of cell function despite the fact that these organelles cannot be directly related to the secretory products of the adrenal cortex. Furthermore, as in other tissues, the mitochondria serve as an index of cell viability, since, when they are irregular in size and non-chromophilic, it can be assumed that the cell is dying.

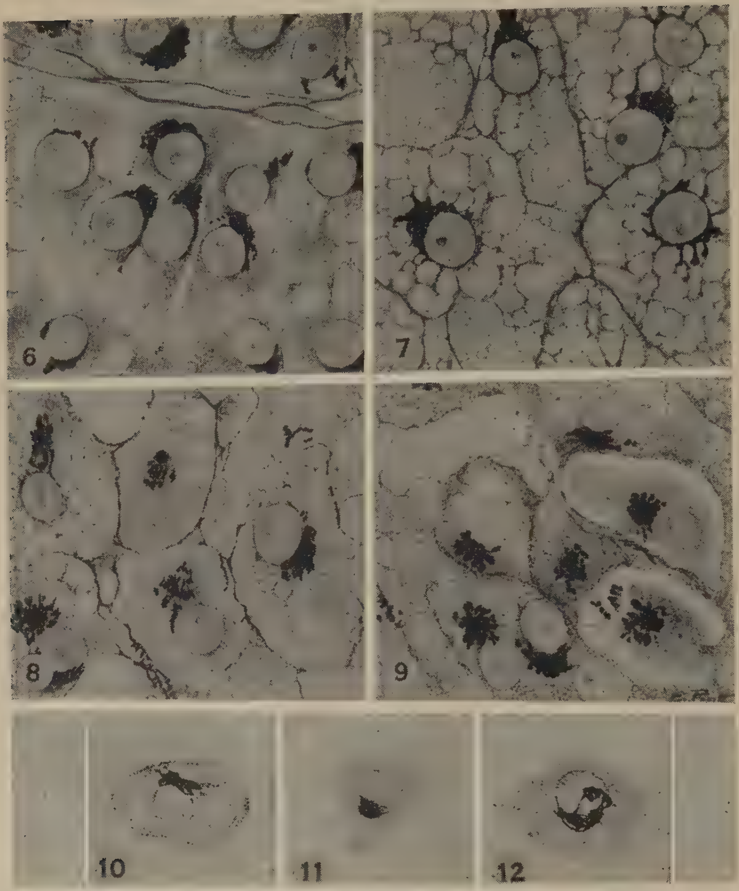
The Golgi Apparatus

The Golgi apparatus in the cells of the adrenal cortex is most successfully demonstrated by the Da Fano silver method because of the large amount of fat which masks the apparatus in osmic acid preparations. By the use of the silver method, the Golgi apparatus can be shown to be a constant component of all the cells of the adrenal cortex. Minor differences are found in the form and appearance of this organelle in different species, but its location is always juxtanuclear.

Bennett[2] described the appearance of the apparatus in the cortex of the cat's adrenal as a network which is compactly arranged in the cells of the zona glomerulosa (FIGURE 6), loose and ramifying in the spongioctyes (FIGURE 7), compact and less well defined in the inner fasciculata (FIGURE 8), and dense and fragmenting in the reticularis (FIGURE 9). He also found that in the glomerulosa of the cat the Golgi substance is constantly located between the nucleus and the nearest capillary, thereby conferring secretory polarity on these cells. Such an orientation, however, was not noticed by Hoerr for the guinea pig,[35] nor by Reese and Moon for the rat.[49] In the rat, Bourne[6] observed two distinct types of Golgi net in the cortical cells—the compact and the diffuse. He believed that the latter represented an hypertrophy of the former, but he failed to describe their zonal location. Bennett, however, felt that the radial extension of the network observable in the spongiocytes is not a true hypertrophy, but rather, a dispersion caused by the presence of crowded lipid droplets.

The observations of Reese and Moon[49] are especially useful in that they describe the Golgi net of rats' adrenals in widely different states of secretory activity. Their observations on normal control rats are in fairly good agreement with those of Bourne. Despite great variation in the form of the Golgi substance, it is clearly most dispersed in the spongiocytes (FIGURE 10). Moreover, the apparatus is smaller and more circumscribed in the cells of the reticularis than in those of the outer fasciculata and glomerulosa. Following hypophysectomy, the Golgi apparatus shrinks and loses most of its radial extensions (FIGURE 11). This response is most marked in the spongiocytes. At only 24 hours after operation, a retraction of the extended processes is evident.

Injections of adrenocorticotropic hormone are reported[49] to produce a striking hypertrophy of the Golgi apparatus, particularly in the cells of the outer fasciculata (FIGURE 12). Heavy, ramifying strands develop, and the central knot becomes much less distinct. An increase in total mass of Golgi



FIGURES 6–12

Drawings of Da Fano silver preparations for the Golgi apparatus, which appears as a blackened knot or network next to the nucleus in cortical cells. FIGURES 6–9, cat's adrenal cortex from Bennett[2]; drawings by Etta Piotti; × 1300. FIGURES 10–12, cells from the zona fasciculata of adrenal cortices of rats; drawings from REESE & MOON.[49]

FIGURE 6. Zona glomerulosa. The blackened channels which comprise the Golgi apparatus are seen as a juxtanuclear cap, lying in each cell between the nucleus and the nearest capillary.

FIGURE 7. Outer fasciculata. The Golgi apparatus sends projections and branches between the lipid "vacuoles."

FIGURE 8. Inner fasciculata. The Golgi apparatus is more compact than in the outer fasciculata, apparently because the lipid spaces are less prominent and the cells less swollen.

FIGURE 9. Zona reticularis. Fragmentation of the Golgi substance and the loss of precise impregnation are to be noted, and is interpreted as manifestations of cell degeneration.

FIGURE 10. Cell from cortex of normal immature rat. The Golgi apparatus appears as a juxtanuclear network with extensions over the nuclear surface.

FIGURE 11. Cell from a rat hypophysectomized 377 days. The cell is much smaller than that illustrated in FIGURE 10, and the Golgi apparatus appears as a shrunken and fused juxtanuclear mass.

FIGURE 12. Cell from an intact rat after the administration of 2 Moon units of adrenotropin. The cell is hypertrophied in comparison to normal, and, likewise, the Golgi net is enlarged and extended.

material is claimed. No significant change is seen in the Golgi body in the inner zones of the cortex. Furthermore, animals that receive injections of adrenotropin for 7 days following removal of the pituitary show that the hormone completely prevents the typical postoperative regression of the Golgi body.

In conclusion, it can therefore be said that the form of the Golgi net is a direct index of the secretory activity of adrenal cortical cells, being compact during inactivity and diffuse and ramifying during marked activity. In addition, most writers believe it is also larger in the spongiocytes than elsewhere.

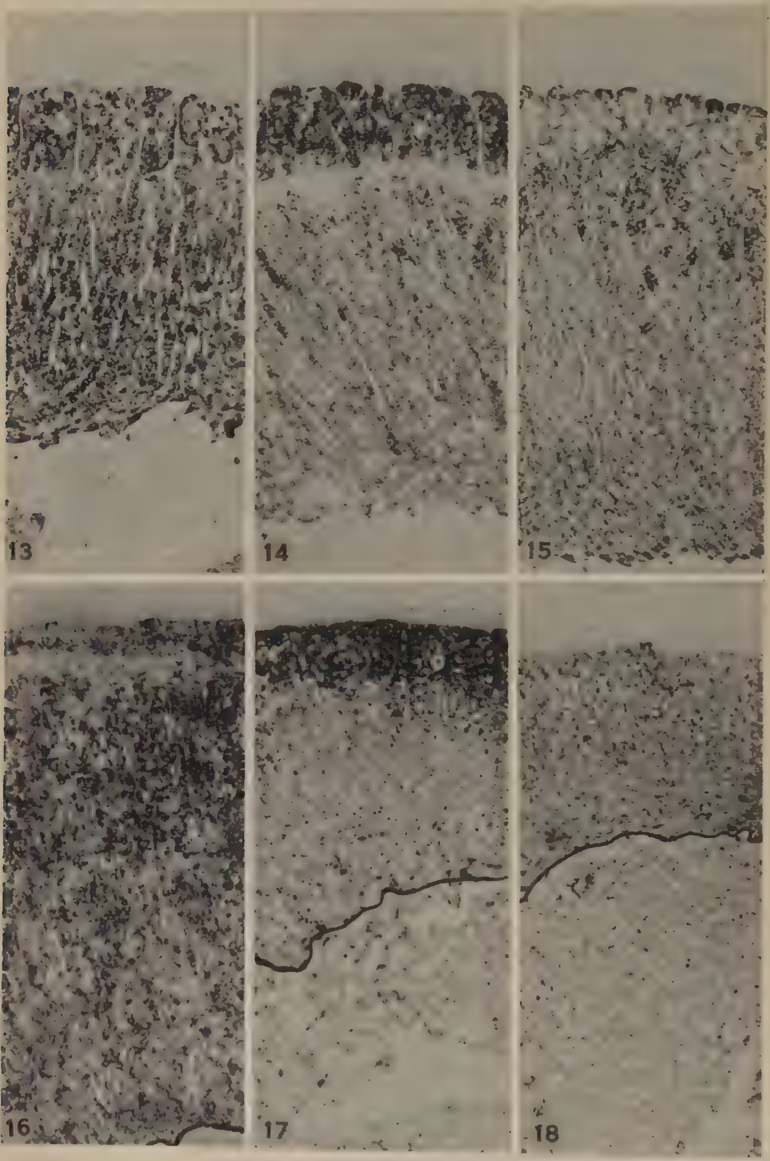
Lipid Droplets

The most prominent cytological feature of the adrenal cortical cells is the presence in the cytoplasm of crowded droplets of fatty material. The lipid droplets are discrete and can be seen in fresh, unfixed tissue. In the unfixed condition, they are spherical and apparently homogeneous, and other shapes which are assumed in fixed material are probably artifacts. The droplets are soluble in organic solvents and, hence, appear as vacuoles after ordinary histological procedures which involve dehydration. It is a common observation, however, that after prolonged treatment with potassium bichromate some of these fatty droplets are preserved and stain in much the same manner as do the mitochondria.[16, 36] Hence, it may be assumed that these particular droplets are closely related to the mitochondria in chemical make-up, *i.e.*, contain phosphatides[36] or are lipoproteins.[8] We will restrict our use of the term "liposome" to refer to droplets which have this staining property. They differ from the simple lipid ones, from which the contents are completely dissolved in mitochondrial preparations (FIGURE 5).

The lipid droplets can be identified in frozen sections by the fact that they take up the fat-soluble, or sudan, dyes. This is a precise cytochemical test. Contrary to general opinion, however, the depth of color of the droplet with the red sudans cannot be related to the chemical constitution thereof, but is attributable to the physical factor of solubility.[43] The lipid droplets also reduce osmium tetroxide, yielding a black product. This is not a specific test for fats, since any unsaturated compound may reduce osmic acid. The use of sudan stains is therefore to be preferred.

In Bennett's study of the cat's adrenal,[2] the locations of the osmiophilic and the sudanophilic substances were essentially identical. The cells of the outer fasciculata, or what he termed the secretory zone, were packed with fairly even-sized droplets, a few of which could be seen outside the cells and apparently entering the sinusoids. None of the other zones of the cat's adrenal were described by him as containing more than a sparse amount of lipid, except for some cells in the reticularis that contained a considerable number of irregular-sized droplets. His illustrations, however, show a sudanophilic outer zone (glomerulosa), which is set off from the lipid-rich fasciculata by a distinct transitional zone (his Figures 4 and 7). We have found that the glomerulosa of the dog's adrenal is conspicuously sudanophilic in comparison to that of the cat (FIGURE 13).

The rat's adrenal, which has been the subject of much study from the standpoint of the distribution of osmiophilic or sudanophilic material,[12, 13, 17, 21, 24, 48, 52, 60] has a very fatty glomerulosa. The lipid droplets in this zone are somewhat larger than those in the fasciculata and are more intensely sudanophilic. The lipid-free zone interposed between these two



FIGURES 13–18

The photomicrographs of FIGURES 13–24 are of 15 μ frozen sections of formalin-fixed adrenals. FIGURES 13–15 are of adrenal cortices of dogs, stained with sudan IV for lipids, no counterstain. From McKIBBIN & DEANE.[45] × 75 FIGURES 16–18, adrenal cortices of rats stained with the Schiff reagent. Ketosteroids can react the Schiff reagent and produce a purple stain. The border between the cortex and the medulla has been drawn in. From GREEP & DEANE.[31] × 135.

FIGURE 13. Cortex of a normal dog, showing three major zones: a conspicuous glomerulosa; a broad fascic-ulata; and a narrow juxtamedullary reticularis. Sudanophilic lipids are abundant throughout the cortex, being somewhat less so in the transitional zone between the glomerulosa and fasciculata.

FIGURE 14. Cortex of a dog on a pantothenic acid deficient diet for a month and suffering from a severe deficiency not moribund, however. A marked deficiency of pantothenic acid induces the adaptation syn-drome.[17] Severe depletion of sudanophilic lipids has occurred from the inner zones, with some lipid remaining in the outer fasciculata; a normal or supranormal amount of lipid remains in the glomerulosa. This illus-trates the "near exhaustion" stage of the adaptation syndrome.

FIGURE 15. Cortex of a dog on a pantothenic acid-deficient diet for about 3 weeks, killed when in coma.

major zones is normally a prominent feature.[54, 60] In the outer fasciculata, the lipid droplets are extremely numerous, whereas, in the inner fasciculata, they are distinctly less numerous and perhaps somewhat smaller in size than in the outer part. The reticularis has droplets which vary enormously in size and shape. They are probably products of fatty degeneration since, as will be shown later, they fail to present the more specific ketosteroid reactions. It should be remarked here that several investigators[13, 54, 57] have described the lipid droplets of the zona fasciculata as fine when the gland is in a state of activity and much coarser during inactivity.

Cytochemistry of the Adrenal Cortex

Reactions for Ketosteroids

The point of greatest interest concerning the lipid droplets is the fact that the adrenal hormones, being lipid-soluble ketosteroids, might be expected to be found dissolved in them. Some of the chemical methods which are used to identify ketosteroids have been adapted to use on tissue sections. Bennett,[2] pioneering in the field of histochemistry applied to ketosteroids, showed that acetone-soluble material present in the adrenal cortex reacts in a manner similar to ketosteroids *in vitro.*

There are, at present, the following cytochemical tests which are significant in identifying ketosteroids in tissues, and, hence, are available for localizing the biologically active hormones of the adrenal cortex.

Phenylhydrazine reaction,[2] *Schiff reaction,*[23] *and semicarbazide reaction.*[2] These three tests all depend on the presence of a ketone or carbonyl group ($c = 0$) in the reactive molecule. Such a group will react with phenylhydrazine to form yellow phenylhydrazones, with leucofuchsin to form a magenta-colored complex (the Schiff or "plasmal" reaction), and with semicarbazide to form semicarbazones. The latter reaction is virtually colorless and is used cytochemically only to prevent other ketone reactions. Ketosteroids, since they possess one or more carbonyl groups, are capable of giving these three tests. It is the lipid droplets of the glomerulosa and the fasciculata which react most strongly with the Schiff reagent (FIGURE 16), although the cytoplasm stains diffusely to a slight degree.

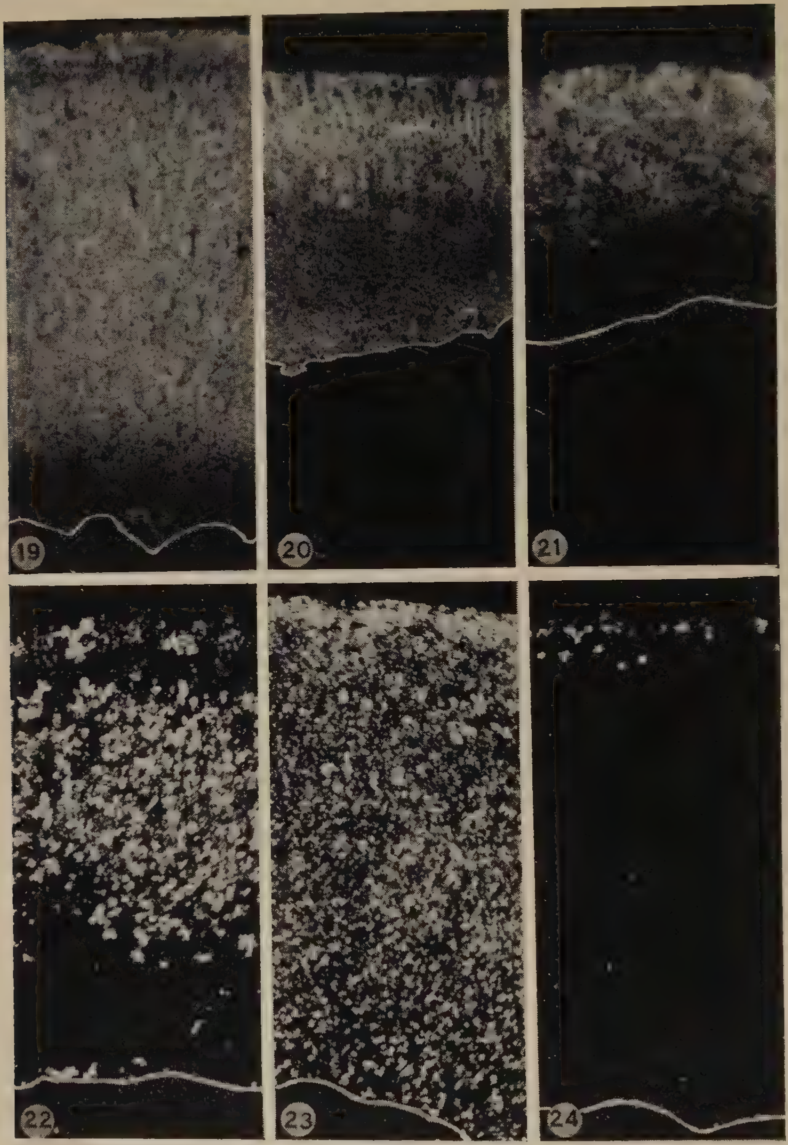
Reichstein's ammoniacal silver reaction.[2, 50] Some ketosteroids possess carbonyl groups active enough to reduce ammoniacal silver solutions. This property is exhibited particularly by the adrenal steroids which possess a carbonyl group at C_{20} and an adjacent hydroxyl at C_{21}. In tissue sections, this reaction is abolished by previous treatment with semicarbazide.[2]

Depletion of lipids from the glomerulosa; fasciculata similar to that in FIGURE 14. FIGURES 14 and 15 illustrate the marked hypertrophy of the fasciculata under the influence of pituitary stimulation.

FIGURE 16. The normal rat adrenal cortex consists of a subcapsular glomerulosa, a transitional zone, a broad fasciculata, and an ill-defined juxtamedullary reticularis. The lipid droplets react strongly with the Schiff reagent in the glomerulosa and in the outer portion of the fasciculata.

FIGURE 17. Cortex of a rat hypophysectomized for 56 days. A marked atrophy of the inner zones has occurred, while the glomerulosa has broadened. Schiff-positive material has all but disappeared from the inner zones, but persists in high concentration in the zona glomerulosa. The maintenance of the glomerulosa suggests that this zone is independent of the pituitary.

FIGURE 18. Cortex of a rat hypophysectomized for 56 days and injected for the final 28 days with 2 mg. desoxycorticosterone acetate daily. The injections have caused further atrophy of the cortex and a complete disappearance of Schiff-positive lipid from the glomerulosa. The disappearance of lipids from the glomerulosa suggests that this zone has undergone disuse atrophy and, therefore, probably secretes desoxy-corticosteroids.



FIGURES 19–24

Adrenal cortices of rats. The border between the cortex and medulla has been drawn in. FIGURES 19–21 illustrate the autofluorescence of the adrenal cortex emitted under ultraviolet light. Autofluorescence characterizes the steroid hormones of the adrenal cortex. From DEANE & GREEP.[16] × 125. FIGURES 22–24 illustrate the distribution and quantity of birefringent lipids in the cortex, birefringence being a property of steroids. From DEANE & SHAW.[19] × 125.

FIGURE 19. The adrenal cortex of a normal rat. A yellowish- or greenish-white autofluorescence characterizes the lipid droplets of the glomerulosa and fasciculata. The transitional zone and the reticularis do not fluoresce.

FIGURE 20. The adrenal cortex of a rat hypophysectomized for 28 days. The marked atrophy of the gland is accounted for by the shrinkage of the inner zones. Only a trace of autofluorescence remains at the juxtamedullary border, while there is an augmented amount in the tissue derived from the glomerulosa. These observations suggest that the fasciculata, but not the glomerulosa, is under pituitary control.

FIGURE 21. The adrenal cortex of a rat hypophysectomized for 136 days. No further atrophy of the cortex

Liebermann-Burchardt reaction. This procedure, involving treatment with a mixture of concentrated sulfuric and glacial acetic acids, produces colored reaction-products with various unsaturated polycyclic compounds.[24b] Unsaturated steroids are prominent among these reactive substances. An adaptation of this reaction for histological purposes, the Schultz test, has long been thought specific for cholesterol, but it now appears to be a general steroid reaction.[5] This reaction has been used successfully on the ovary,[22, 24a] but to our knowledge has not been applied to the adrenal.

Birefringence. When observed in polarized light, droplets containing ketosteroids appear bright on a dark background. This property, which is caused by the radial symmetry of the steroid molecules in liquid spherocrystals,[43] is an expression, therefore, of the molecular pattern assumed by this class of compounds. Bennett[2] adopted the further refinement of pre-treating frozen sections with digitonin, which forms insoluble, anisotropic esters with cholesterol[41] and other β-steroids, thus producing more birefringence.

An important aspect of the study of birefringence in adrenal sections is the consideration of particle size. Accumulating evidence suggests that fine particles characterize active secretion, whereas large particles indicate inactivity or storage.[19, 21, 65]

Autofluorescence. The biologically active ketosteroids, including those of the adrenal cortex, exhibit fluorescence.[5, 50] This property is a result of the excitation of electrons in the molecule by the absorption of light quanta. The lipid droplets of the glomerulosa and fasciculata in adrenal sections viewed microscopically with ultraviolet light emit a yellowish or greenish-white fluorescence[16, 34] (FIGURE 19).

Solubility. Steroids are soluble in cold acetone. This property is utilized negatively in the evaluation of the above tests, since any material which retains its reactivity following such extraction cannot be considered steroidal.

In summarizing this section, it should be said that, although ketosteroids react positively with all of these procedures, no one of the tests is specific for these compounds. Thus, convergent evidence obtained by the simultaneous use of several or all of these methods is a more trustworthy indication of the presence of ketosteroids than is the result of any single reaction. Furthermore, no single class of substances, other than ketosteroids, exhibits positive reactions with the entire battery. Positive results from this series of reac-

---

has occurred, but the inner zones are now entirely depleted of autofluorescent material. The glomerulosa, on the other hand, is even broader and also contains more fluorescent lipid than at 28 days.

FIGURE 22. Normal adrenal cortex of an adult rat. Birefringent particles of varying size characterize the glomerulosa and a broad segment of the fasciculata. The results of Weaver and Nelson[65] indicate that coarse particles represent storage material and that fine particles represent hormone in the process of release.

FIGURE 23. Cortex of a rat that had experienced a reduction in food supply for approximately 2 weeks. This figure illustrates the "resistance" phase of Selye's adaptation syndrome,[58] with the whole cortex filled with birefringent material. Many fine particles may be seen.

FIGURE 24. Cortex of a rat fed a thiamine-deficient diet for 4 weeks. Like pantothenic acid deficiency, a thiamine deficiency induces a typical adaptation syndrome. Complete depletion of birefringent steroids from the fasciculata has occurred, although the glomerulosa retains some. The restriction of the changes in quantity of steroids during the adaptation syndrome principally to the zona fasciculata leads us to believe that this zone secretes the 11-oxy-corticosteroids, the secretion of which is particularly augmented in conditions of stress.

tions indicate the presence of lipid-soluble, carbonyl-containing molecules, the anisotropicity and autofluorescence of which are similar to those of known ketosteroids. It seems unlikely that other possible reactants would occur superimposed in such a fashion as to give positive results by all these tests.

The experimental material which will be reviewed below adds further circumstantial evidence favoring the thesis that the reactive materials are ketosteroids, since, in physiological situations where the production of hormones rises or declines, the tests wax and wane in a parallel fashion.

Experimental Modification of the Ketosteroid Content of the Adrenal Cortex

Hypophysectomy. The combined morphological and cytochemical changes which occur in the adrenal cortex after removal of the pituitary are of extraordinary interest. The adrenal cortex was early found to undergo such severe atrophy that it seemed unlikely that any part of its function could be retained. Enigmatically, however, hypophysectomized animals survived, while totally adrenalectomized rats succumbed unless given supplementary sodium chloride.

In the adrenal cortex of the rat after hypophysectomy, the fasciculata and reticularis atrophy the most severely.[16] The cells are reduced in size and eventually in numbers, so that, after 2 months, the zona fasciculata can scarcely be said to exist and cannot be distinguished from the reticularis. The glomerulosa broadens as the fasciculata shrinks and may increase from about 6 cell layers (normal) to 14 to 18 cell layers (compare FIGURES 19, 20, and 21). This increase in depth of the glomerulosa is due to a rearrangement of the cells on the periphery of a shrinking sphere, as well as, probably, to the addition of new cells.

We have followed the redistribution and changes in the reactions of the lipid droplets in the cortex of rats at intervals over a period of 4 months after removal of the pituitary. Along with the preservation of sudanophilia in the widened glomerulosa, we find that ketosteroids are likewise retained, as is indicated by the presence of acetone-soluble, birefringent, autofluorescent (FIGURES 20 and 21), and Schiff-positive (FIGURE 17) material. The transitional zone becomes definitely wider. With the slow leaching-out of lipid droplets from the shrinking fasciculata, the Schiff-positive and birefringent material also diminishes, but the natural fluorescence is lost most rapidly (FIGURES 20 and 21). Thus the fasciculata gradually loses the principal histochemical reactions indicative of the ketosteroids.

That the glomerulosa is not a conspicuous target organ of the anterior lobe of the pituitary is supported by the following evidence: after hypophysectomy, the cells maintain a healthy appearance; the thickening of the glomerulosa indicates that this zone is attempting to maintain its preoperative volume; and the persistence of Schiff-positive, birefringent, and autofluorescent material at normal or more than normal levels for at least 4 months is presumptive evidence of a continuing formation of ketosteroids. Since an array of physiological data indicates the continued secretion of salt-conserving corticosteroids following hypophysectomy,[37, 61] these observations suggest that the glomerulosa is the source of such hormones, and that it is independent of the pituitary. (See, however, conflicting evidence for the human.[3a])

The zona fasciculata, on the other hand, appears to be completely dependent on the hypophysis. The activity of the pituitary is required both for maintenance of cell size and for the formation of secretions in this zone. Since the 11-oxy-steroids, which are concerned with gluconeogenesis[38, 44] and the release of antibodies,[67] are apparently not secreted following hypophysectomy, their origin may, therefore, be attributed to the fasciculata.

Stress. The rôle of the adrenal cortex in the adaptation of the body to noxious agents or to environmental situations which create an unusual demand for energy has been intensively explored by Selye.[57, 58] The mechanism by which the adrenal produces such adaptation still remains somewhat obscure. It is widely accepted, however, that a heightened activity of the adrenal cortex is involved. The evidence is two-fold: (1) injections of adrenal extracts or 11-oxy-steroids better enable an animal to survive a stress;[38] and (2) in stress, the adrenal undergoes morphological and cytochemical modifications which constitute presumptive evidence of a rise in secretory activity.

Our concern here is with the latter evidence. It is of major importance that the glomerulosa fails invariably to take any great part in these modifications. The fasciculata, on the other hand, is subject, not only to an increase in volume (FIGURES 14 and 15), but to extreme alterations in lipid content and distribution. The adrenal changes which characterize the three stages of the adaptation syndrome, as defined by Selye,[58] are as follows: The immediate reaction to an alarming stimulus is an hypertrophy of the fasciculata with accompanying lipid depletion (the "alarm reaction"). During the "resistance" phase the lipid reaccumulates in the fasciculata and may be present in supranormal amounts (FIGURE 23). In the "exhaustion" phase, the fasciculata undergoes maximum hypertrophy and lipid depletion becomes extreme (FIGURES 14 and 24).

Observations on the effects of stress on the adrenal cortex have generally been confined to the changes in distribution of osmiophilic or sudanophilic material.[12, 13, 21, 24, 35, 54, 55, 68] That these methods alone are inadequate means of determining adrenal secretory activity has only recently been clearly recognized. The studies of Popják,[48] and of Deane and associates indicate that a complete parallelism does not obtain between sudanophilia and the more specific ketosteroid reactions.

Popják[48] studied the first phase of the adaptation syndrome by examining the adrenals of rats during the 48 hours following a traumatic injury. He performed parallel chemical and cytochemical analyses. Frozen sections stained with sudan IV were compared with adjacent sections treated with phenylhydrazine and others examined with polarized light. At 24 hours after injury, the depletion of sudanophilic and birefringent lipids from the fasciculata was associated both with an increase in the intensity of the yellow phenylhydrazine reaction, and with a widening of the zone showing the reaction. Chemically, he found a reduction of cholesterol during the same period. Thus, during the "alarm reaction," with a reduction of total lipid as demonstrated by sudanophilia, birefringence, and total steroid, there was a rise of free ketones as demonstrated by the phenylhydrazine reaction.

Studying the whole course of the adaptation syndrome in rats[17, 19] and dogs[45] subjected to several different nutritional inadequacies, Deane and co-workers have observed a rise in sudanophilia in the "resistance" phase, followed by a decrease in the "exhaustion" phase which begins at the juxtamedullary border and continues peripherally (FIGURE 14). During the early part of the resistance phase, there is a notable increase in birefringence in the fasciculata (especially of fine particles indicating secretion), which is maintained for some time after the fall in sudanophilia begins (FIGURE 23). At the time of exhaustion, birefringence also drops (FIGURE 24). In this study, birefringence seemed to serve as a more significant index of steroid content and secretion than sudanophilia. This is probably because sudanophilia characterizes both triglycerides and steroids, whereas birefringence probably signifies only the latter. Interestingly enough, Oleson and Bloor[47] found, chemically, a decline of triglycerides in rabbit adrenals while the steroids continued to rise, during the "resistance" phase.

It has been well established that, under conditions of stress, an hypertrophy of the cortex and an increase and subsequent depletion of its lipid are associated with increased cortical secretion. This is indicated by the fact that the corticosterones afford added protection against alarming agents and also by the appearance of cortin-like substances in the urine of patients experiencing physical stress.[66] The adrenal changes may be attributed to an increased secretion of adrenotropin. Using adrenotropin, Bergner and Deane,[3a] have simulated the "alarm reaction." In confirmation of Popják's observations, there was a decrease in the amount of sudanophilic and birefringence material. The reduction in birefringence was accomplished, principally, by the loss of the coarse particles, whereas the residual particles were of the fine variety. Accompanying these changes, there was an increase in intensity of the Schiff reaction and of autofluorescence. It would seem, therefore, that these changes are associated with the active release of hormone from the fasciculata. Sayers and associates,[56] Levin,[42] and others have demonstrated chemically a similar fall in adrenal steroids after injecting adrenotropin.

Disuse Atrophy. It is a common endocrinological observation that a gland ceases to function and becomes atrophic if the hormone which it produces is artificially provided. In no instance have experiments of this nature been more revealing nor yielded more definite information than those concerned with the adrenal glands.

1. 11-Oxygenated corticosteroids. It has been amply demonstrated that injections of adrenal cortical extract or of the 11-oxygenated corticosteroids bring about a reduction in the size of the adrenal cortex.[39] Examined histologically, such glands show an atrophy confined to the inner zones, and the glands bear a remarkable resemblance to those of hypophysectomized rats. Deane, Thorn, and associates[20] have made a cytochemical study of the adrenal cortex after injections of corticosterone. The distribution pattern of the sudanophilic material is altered in exactly the same manner as that produced by hypophysectomy: the individual sudanophilic droplets and birefringent particles in the fasciculata become larger as the zone atrophies. In contrast to these changes in the fasciculata, the lipids of the glomerulosa are unaffected.

Ingle[37] and others have commented on the possibility that the shrinkage of the fasciculata may be another instance of compensatory or disuse atrophy. This interpretation is so thoroughly indicated by circumstantial evidence that we feel justified in accepting it. Although the mechanism involved has not been entirely proven, the likelihood is very great that the corticosterones operate by suppressing the production of adrenotropin.

2. *Desoxy-corticosteroids.* The adrenals of normal rats treated with desoxycorticosterone acetate show a very different response from that seen after injections of 11-oxy-steroids. Carnes *et al.*[10] and Sarason[54] have observed that there is a disappearance of lipid from the glomerulosa following the prolonged administration of desoxycorticosterone acetate. We have found, after 7 to 12 days' treatment with desoxyocrticosterone acetate, that the glomerulosa is less intensely sudanophilic and its autofluorescence is much less conspicuous. By 28 days, there are no longer any sudanophilic droplets in the glomerulosa, nor could we find a trace of ketosteroid by any test.[31]

Similar injections were made into hypophysectomized rats. The treatment was started 28 days after the operation, when the lipids of the fasciculata were greatly depleted and the glomerulosa was considerably thickened and laden with sudanophilic, birefringent, autofluorescent, and Schiff-positive material. After 28 days of treatment, the glomerulosa was completely negative to all these tests. Since the fasciculata was already atrophic, this latter treatment produced a cortex that was unreactive throughout (FIGURE 18).

These results are complementary to those produced by injections of corticosterone, and we attribute them to a similar phenomenon, namely, disuse atrophy. However, the mechanisms involved cannot be similar. Desoxycorticosterone does not operate through the pituitary to shut off the stimulus to the glomerulosa, since the reaction occurs in hypophysectomized animals. The mechanism which stimulates and regulates the glomerulosa is unknown. We can speculate that the secretory activity of the glomerulosa may be regulated by the concentration of one or more of the electrolytes in the body fluids, much as that of the parathyroid glands appears to be regulated by the blood level of ionic calcium.[30]

Using larger doses of desoxycorticosterone than those in our experiments, many authors have reported severe atrophy of the fasciculata as well.[10, 11, 59] Following such treatment, the osmic acid preparations of Carnes and associates[10] do not show any change in the relative amount of lipid in this zone, but further cytochemical studies are needed on such material to determine whether hormone production is normal (cf.[31]).

Ascorbic Acid

The known abundance of vitamin C in the adrenal cortex and the recent studies concerning its cyclic rise and fall in relation to cortical activity make the cytochemical investigation of this material highly desirable. Ascorbic acid is the only known biological substance having the capacity to reduce silver nitrate at low pH. Histochemical utilization of this property permits the precise histological localization of this important acid. The acid silver nitrate is variously applied: (1) by perfusion[28]; (2) to unfixed, frozen sections[7]; or (3) in alcohol, to a freshly excised block of tissue.[7] In our hands, the last named method has been most successful. Following immersion in the silver nitrate-alcohol, the block is treated with acidified sodium thiosulfate, and then imbedded in paraffin, sectioned, and counterstained lightly with a nuclear stain.[18] The only disadvantage of these histochemical methods is that vitamin C may be present in an oxidized form and, therefore, not exhibit its strong reducing power. Thus, a positive reaction is certainly significant, but a negative one need not be.[28]

A number of observations on the distribution of ascorbic acid in the adrenal cortex have been published. The papers of Bourne[7] and Leblond and co-workers[26, 27, 28, 40] are the most notable. In the normal adrenal cortex of a variety of species, a precipitate of silver is seen to occur in all of the cells, distributed as fine granules in the zona glomerulosa (FIGURE 2) and as coarse ones in the fasciculata and reticularis (FIGURE 3). It is generally reported that there is less ascorbic acid in the glomerulosa than in the rest of the cortex.[26] Moreover, granules of reduced silver may also occur in the sinusoids, a phenomenon which suggests the possible secretion of ascorbic acid.

The precise intracellular localization of ascorbic acid is difficult to ascertain, because it probably exists in solution within the cell and may migrate before fixation is completed. In our experience, the speed of immersion of the block in the reagent clearly affects the distribution. Uniform distribution characterizes immediate fixation, and clumping in the Golgi region occurs when fixation is more leisurely. These results suggest the possibility of post-mortem migration.[18] For this reason, the many studies describing localization of vitamin C on the mitochondria[27] or within the Golgi apparatus[8, 27] seem to us of little significance.

There are now several chemical studies showing a concomitant decline in ascorbic acid and cortical steroids in sundry conditions involving stress.[29, 56] A cytochemical study has likewise demonstrated parallel disappearance of lipids and ascorbic acid from the adrenal cortex of the guinea pig in scurvy.[4] Cytochemical studies of the adrenal in other experimental conditions are now greatly indicated (cf.[18]).

The History of the Cells of the Adrenal Cortex

The hypothesis that the cells of the adrenal cortex arise from the capsule and migrate centripetally in the cortical fascicles to die at the medullary border is widely held.[2, 35, 71] Through restatement, this idea has become entrenched in endocrine literature and thought. Most writers state or imply that the cells undergo a single cycle of secretion as they migrate inward, and on this basis each zone of the cortex represents one stage of the secretory cycle. Bennett[2] utilized this concept as a basis for interpreting the distribution of ketosteroids in the cortex in a histophysiological manner.

Actually, this concept of cell migration, which for brevity we will call the "escalator" theory, is founded on two assumptions, neither of which can be regarded as proven: (1) that mitosis occurs solely in the outer regions of the cortex; and (2) that cell death occurs almost entirely in the zone bordering the medulla.

Cell Division. In the adrenal cortex, cell division occurs predominantly in two localities, namely, in the glomerulosa and in the outer region of the fasciculata. Zwemer and associates[71] have been inclined to consider only the former location in their enthusiasm for the "escalator" theory. Hoerr[35] and Nathanson and Brues,[46] studying stimulated adrenals, observed mitotic figures throughout the cortex, but essentially disregarded those in the deeper layers of the cortex because of their belief in a steady inward flow or migration of the cells. Recently, however, with the repeated observation of the occurrence of mitosis in all sections of the cortex, workers have gradually come to doubt the acceptability of the "escalator" theory.

Two helpful studies of the distribution of mitotic figures are available. Baxter[1] found the number of arrested figures per 10,000 cells at 16 hours after the injection of colchicine to be:

capsule	52
zona glomerulosa	116
zona fasciculata	157
zona reticularis	66

Since colchicine may itself serve as an alarming stimulus, the exact relative distribution of mitotic figures as given here may not be an index of the distribution in the normal cortex. Nevertheless, it indicates the capacity for cell division in all the zones of the cortex.

Vaccarezza,[62] studying cell division in normal adrenals and those subjected to direct lesions, concluded that there is local replacement of cells anywhere in the cortex whenever death occurs in isolated cells. If, on the other hand, there is an extensive lesion, mitoses may occur at some distance from it, with the result that the area of injury is filled in by centripetal proliferation. Recently we[32] have studied the regeneration of cortical tissue following severe enucleation of the adrenal. Mitoses occur throughout the regenerating mass, rather than being limited to the subcapsular region. However, the new tissue appears to be derived entirely from cells which once lay in the glomerulosa. Since the new gland is capable of secreting both "salt" and "sugar" hormones, it seems clear that transformation of glomerulosa-derived cells into fascicular cells can take place under these extreme circumstances. Suffice it to say that under normal circumstances the distribution of mitotic figures does not support the assumption of continuous migration of cells from the capsule to the medullary border.

Cell Death. The reticular zone has been universally described as an area of cell destruction and death.[2, 35, 70] The evidence favoring this belief is impressive, and we do not deny that much cell destruction occurs here. We only submit that an obsession with the concept of cell migration has too often led histologists to believe cells die only in this zone. Many of the signs of so-called abnormality may merely be a response to the relatively poor quality of the blood supply here. A similar condition obtains at the center of the liver lobule, where the supply of oxygen has been shown to be low. Here the cells contain lightly staining, fragmenting mitochondria and a poorly defined Golgi apparatus, and, also, frequently experience fatty infiltration.[15] Perhaps, when the adrenal circulation speeds up in the alarm reaction and these cells receive blood with more oxygen and essential food-stuffs, they may "recover." Thus, Flexner and Grollman[25] doubted the degeneracy of this zone when they found that it responded to a stress, concomitantly with the inner fasciculata, by increasing in width.

Bennett[2] used supravital trypan blue to detect dying cells.[14] Although he found unquestionable evidence of cell death in the reticularis, the point of great moment, for the purpose of the present discussion, is the large number of dying cells which he found in the other zones, particularly among the spongiocytes.

Studies Employing Intravital Trypan Blue. From histological examination of the adrenal cortex of several different species, Zwemer and coworkers[71] concluded that cells were constantly being contributed to the glomerulosa from what they called indifferent fibroblast-like cells located in the adrenal capsule. Knowing that fibroblasts ingest a small amount of trypan blue, Salmon and Zwemer[53] studied the adrenals of rats receiving intravital injections in the hope of marking the capsular cells so that their subsequent history could be followed. The trypan blue was first taken up by macrophages in the capsule, but, after 3 days, began to appear in the "fibroblast-like" cells which they believed gave rise to parenchymal cells. These investigators described the inner border of the vitally stained zone as migrating progressively deeper and deeper into the cortex, until, at 20 to 30 days, the zone of staining had reached the reticularis. In the analysis of these results, no distinction appears to have been drawn between the glandular cells containing fine particles of trypan blue and dye-laden cells lining the sinusoids. Furthermore, neither the length of time the dye remained in the blood stream nor the migratory habits of phagocytic endothelial cells were taken into consideration. Nevertheless, this study has been looked upon generally as providing of cell migration.

A preliminary reinvestigation of this work[9] has brought out the fact that the location of the dye in the cortex bears no relation whatever to the time elapsed after injection. Recently, Baxter[1] has carried out an even more detailed study and has found not the slightest evidence for the centripetal migration of cortical cells. The dye was phagocytized by individual cells in the capsule, glomerulosa, and reticularis within 24 hours. At 4 to 5 days, the dye also appeared in the zona fasciculata, but only in macrophages. He emphasized that the concentration of the dye in the blood stream did not fall for 6 days following an injection. Weatherford has made similar observations and come to the same conclusions.[64]

Tissue Culture Studies. Williams[69] utilized a moist chamber in the rabbit's ear for observing the behavior of transplants of isolated fragments of capsule, of glomerulosa, and of fasciculata from adrenal cortex. The cells which arose from capsular fragments were typical fibroblasts. The glomerulosal grafts became well vascularized and showed mitoses and cyclic secretory activity which could be followed due to the refractile property of the cytoplasmic droplets. The fasciculata grafts did not become vascularized nor exhibit mitosis and were slowly reduced in size. The observation of importance for our purpose, however, is that, in the course of 8 months, there was no observed instance of the transmutation of a glomerulosal cell into the fasciculata type, nor *vice versa.*

Evidence from the Location of the Reticulum. Whether intercellular connective tissue exists in the cortex is important from the standpoint of the migration of the parenchymal cells. Hoerr described few reticular fibers penetrating between the cells of the fascicles and used this as an argument for the easy motility of the individual cells.[35] The reticulum illustrated by Gruenwald and Konikov[33] shows, however, a profusion of fibers standing as barriers across the supposed paths of migration.

Summary

Sufficient differences in the form and chromophilia of the mitochondria and of the Golgi apparatus exist in active and inactive cortical cells to justify the belief that a definite correlation obtains between the character of these organelles and the state of secretory activity of the cells.

Ascorbic acid occurs throughout the adrenal cortex. It appears as fine granules in the cells of the glomerulosa and as coarse and crowded granules in those of the fasciculata and reticularis

Ketosteroids may be characterized in frozen sections of the adrenal cortex by their reactions with sudan dyes, phenylhydrazine, semicarbazide, the Schiff reagent, and ammoniacal silver nitrate; furthermore, they are birefringent, exhibit a greenish-white autofluorescence, and are acetone-soluble.

The lipid droplets in the glomerulosa and the outer part of the fasciculata appear to contain ketosteroids by the above tests. The droplets in the cells of the inner part of the fasciculata and of the reticularis are presumably composed principally of triglycerides, since they are sudanophilic but exhibit only variable traces of the other steroid reactions.

In the hypophysectomized rat, the lipids and ketosteroids of the zona fasciculata are slowly lost, the mitochondria become smaller, and the Golgi knot more compact. The glomerulosa retains its sudanophilic, Schiff-positive, birefringent, and autofluorescent lipid droplets and becomes thicker as the adrenal atrophies.

In the adaptation syndrome there are pronounced changes in the adrenal cortex, but it appears that the glomerulosa plays no part in this reaction. The fasciculata displays a series of prominent morphological and cytochemical changes. In severe and prolonged stress, the mitochondria of the zone become swollen, less chromophilic, and eventually vesiculated, and the Golgi net becomes even larger and more extended than normal. The ketosteroid content is initially decreased, increases to above normal amounts during the "resistance" phase, then declines, and disappears terminally. The fasciculata enlarges after injections of adrenotropin; its ketosteroid reactions initially decline and later are supranormally augmented, while those of the glomerulosa remain unchanged.

In normal and hypophysectomized rats injected with desoxycortico-

sterone acetate the lipid and ketosteroid content of the glomerulosa slowly declines to extinction. Treatment with this substance does not alter in any way the reactions of the fasciculata. Conversely, after the administration of adrenal cortical extract or of 11-oxy-corticosteroids, the ketosteroid droplets of the fasciculata disappear.

On the basis of the observations that (1) cell division and cell death occur throughout the cortex, (2) *intravitam* trypan blue fails to indicate translocation of cells, and (3) grafts of the adrenal capsule, glomerulosa, and fasciculata fail to undergo cytomorphosis into the other cell types, it may be concluded that the cortical cells generally arise, secrete, and die in their respective zones.

Our data support the following over-all view of the structural and functional interrelationship of the adrenal cortex. The zona glomerulosa, a seemingly autonomous region, in the rat at least, secretes desoxy-corticosteroids, which regulate fluid and electrolyte balance; the fasciculata secretes 11-oxy-corticosteroids, which are concerned with gluconeogenesis, resistance to stress, and the physiological mechanism of antibody production. The latter zone appears to be controlled entirely by the anterior pituitary.

Literature Cited

1. BAXTER, J. S. 1946. J. Anat. **80:** 139.
2. BENNETT, H. S. 1940. Am. J. Anat. **67:** 151.
3. BENNETT, H. S., & L. KILHAM. 1940. Anat. Rec. **77:** 447.

3a. BERGNER, G. E. & H. W. DEANE. 1948. Endocrinology **43:** 240.

4. BESSEY, O. A., M. L. MENTEN, & C. G. KING. 1934. Proc. Soc. Exp. Biol. & Med. **31:** 455.
5. BIERRY, H. & B. GOUZON. 1936. C. R. Acad. Sci. **202:** 686.
6. BOURNE, G. 1934. Austr. J. Exp. Biol. & Med. Sci. **12:** 123.
7. BOURNE, G. 1935. Austr. J. Exp. Biol. & Med. Sci. **13:** 113.
8. BOURNE, G. 1942. *Cytology and Cell Physiology:* 99 Oxford Univ. Press.
9. CALMA, I. & C. L. FOSTER. 1943. Nature **152:** 536.
10. CARNES, W. H., C. RAGAN, J. W. FERREBEE, & J. O'NEILL. 1941. Endocrinology **29:** 144.
11. CASTILLO, E. B. DEL & C. E. RAPELA. 1945. Rev. Soc. Argentina Biol. **21:** 338.
12. DALTON, A. J. 1944. J. Nat. Cancer Inst. **5:** 99.
13. DALTON, H. J., E. R. MITCHELL, B. F. JONES, & V. B. PETERS. 1944. J. Nat. Cancer Inst. **4:** 527.
14. DARLINGTON, J. M. 1937. Anat. Rec. **67:** 253.
15. DEANE, H. W. 1944. Anat. Rec. **88:** 39.
16. DEANE, H. W. & R. O. GREEP. 1946. Am. J. Anat. **79:** 117.
17. DEANE, H. W. & J. M. MCKIBBIN. 1946. Endocrinology **38:** 385.
18. DEANE, H. W. & A. MORSE. 1948. Anat. Rec. **100:** 127.
19. DEANE, H. W. & J. H. SHAW. 1947. J. Nutrition **34:** 1.
20. DEANE, H. W. G. W. THORN, G. BERGNER, P. H. FORSHAM, & G. T. PRUNTY. Unpublished results.
21. DEANESLY, R. 1931. Am. J. Anat. **47:** 475.
22. DEMPSEY, E. W. & D. L. BASSETT. 1943. Endocrinology **33:** 384.
23. DEMPSEY, E. W. & G. B. WISLOCKI. 1944. Endocrinology **35:** 409.
24. DOSNE, C. & A. J. DALTON. 1941. Anat. Rec. **80:** 211.

24a. EVERETT, J. W. 1945. Am. J. Anat. **77:** 293.

24b. FIESER, L. F. 1937. *The Chemistry of Natural Products Related to Phenanthrene* (second edition). Reinhold. New York.

25. FLEXNER, L. B. & A. GROLLMAN. 1939. Anat. Rec. **75:** 207.
26. GIROUD, A. & C. P. LEBLOND. 1935a. Arch. Anat. Micr. **31:** 111.
27. GIROUD. A. & C., P. LEBLOND. 1935b. Bull. Hist. Appl. **12:** 49.
28. GIROUD, A. & C. P. LEBLOND. 1936. Nature **138:** 247.
29. GIROUD, A. & A. R. RATSIMAMANGA. 1942. Actual. Scient. & Indust. **921:** III.

30. Greep, R. O. 1948. The Hormones **1**: 225. Academic Press.
31. Greep, R. O. & H. W. Deane. 1947. Endocrinology **40**: 417.
32. Greep, R. O. & H. W. Deane. 1949. Endocrinology **45**.
33. Gruenwald, P. & W. M. Konikov. 1944. Anat. Rec. **89**: 1.
34. Hamprel, H. 1934. Virch. Arch. Path. Anat. & Physiol. **292**: 1.
35. Hoerr, N. L. 1931. Am. J. Anat. **48**: 139.
36. Hoerr, N. L. 1936. Anat. Rec. **66**: 317.
37. Ingle, D. J. 1942. Endocrinology **31**: 419.
38. Ingle, D. J. 1944. Endocrinology **34**: 191.
39. Ingle, D. J., G. M. Higgins, & E. C. Kendall. 1938. Anat. Rec. **71**: 313.
40. Leblond, C. P. & W. U. Gardner. 1938. Anat. Rec. **72**: 119.
41. Leulier, A. & L. Revol. 1930. Bull. Hist. Appl. **7**: 241.
42. Levin, L. 1945. Endocrinology **37**: 34.
43. Lison, L. 1936. *Histochimie Animale:* 189. Gauthier-Villars. Paris.
44. Long, C. N. H. 1942. Endocrinology **30**: 870.
45. McKibbin, J. M. & H. W. Deane. Unpublished results.
46. Nathanson, I. T. & A. M. Brues. 1941. Endocrinology **29**: 397.
47. Oleson, M. C. & W. R. Bloor. 1941. J. Biol. Chem. **141**: 349.
48. Popják, G. 1944. J. Path. & Bact. **56**: 485.
49. Reese, J. D. & H. D. Moon. 1938. Anat. Rec. **70**: 543.
50. Reichstein, T. & C. W. Shoppee. 1943. Vitamins and Hormones **1**: 345.
51. Rogoff, J. M. 1932. *Special Cytology* **2**: 871. (Second edition.) Hoeber. New York.
52. Rokhlina, M. R. 1940. C. R. (Doklady) Acad. Sci. U. R. S. S. **27**: 500.
53. Salmon, T. N. & R. L. Zwemer. 1941. Anat. Rec. **80**: 421.
54. Sarason, E. L. 1943. Arch. Path. **35**: 373.
55. Sayers, G., M. A. Sayers, E. G. Fry, A. White, & C. N. H. Long. 1944. Yale J. Biol. & Med. **16**: 361.
56. Sayers, G., M. A. Sayers, T.-Y. Liang, & C. N. H. Long. 1945. Endocrinology **37**: 96. 1946. Endocrinology **38**: 1.
57. Selye, H. 1937. Endocrinology **21**: 169.
58. Selye, H. 1946. J. Clin. Endocrinol. **6**: 117.
59. Selye, H. & C. Dosne. 1940. Proc. Soc. Exp. Biol. & Med. **44**: 165.
60. Simpson, M. E., H. M. Evans, & C. H. Li. 1943. Endocrinology **33**: 261.
61. Swann, H. G. 1940. Physiol. Rev. **20**: 493.
62. Vaccarezza, A. J. 1944. Arch. Soc. Argentina Anat. Normal y Patol. **6**: 401.
63. Vaccarezza, A. J. 1945. Medicina **5**: 425; **6**: 46.
64. Weatherford, H. L. Personal communication.
65. Weaver, H. M. & W. O. Nelson. 1943. Anat. Rec. **85**: 51.
66. Weil, P. G. & J. S. L. Browne. 1940. J. Clin. Invest. **19**: 772.
67. White, A. & T. F. Dougherty. 1946. Ann. N. Y. Acad. Sci. **46**: 859.
68. Whitehead, R. 1942. J. Path. & Bact. **54**: 169.
69. Williams, R. G. 1945. Am. J. Anat. **77**: 53.
70. Wolfensohn, L. 1941. C. R. (Doklady) Acad. Sci. U. R. S. S. **31**: 644.
71. Zwemer, R. L., R. M. Wotton, & M. S. Norkus. 1938. Anat. Rec. **72**: 249.

THE ADRENAL CORTEX AND ITS TUMORS*

By George W. Woolley
Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

It is evident, from cases taken from the literature, that in man a remarkable association between conditions of the adrenal cortex and genital and sexual changes has been recognized for many years. Males have been much less often affected than females.

The findings have been varied, both as to the age at which the symptoms appear, and as to the abnormalities and changes in primary and secondary sex characters which occur.

Recently, a new experimental approach has been made to tumors of the adrenal cortex in laboratory animals. The present report concerns studies made with the laboratory mouse. Since mice have a great similarity to man in their bodily structures, mice can conveniently be used in large numbers to establish principles which in turn may be modified for use with man. The adrenal tumor problem, a problem which has proven difficult to solve in man, may be one of these.

The Adrenal Cortex. It is interesting to approach the problem of the adrenal cortex by a review of the adrenal glands from a phylogenetic standpoint. Tissues corresponding to the interrenal body or the cortex of the mammalian adrenal have not been demonstrated anatomically in animals below the cyclostoma (hags and lampreys). The homologue of the chromophil or medullary tissue has been demonstrated in a number of lower invertebrates. In the anamniota (amphibia, fishes, and cyclostomes), the compact union of chromophil and interrenal tissue has not occurred and these tissues are represented by a number of bodies. Amphibia are intermediate in that there is a union of interrenal and chromophil tissue into a single organ without penetration of the latter tissue to assume a true medullary position. The adrenals of the bird show an interlacement of the interrenal and medullary tissues. It is only in the mammals that we find the interrenal tissue as a cortex surrounding a medulla composed of chromophil tissue. In man and in higher vertebrates, the adrenal in cross section is seen to consist of two parts, a soft, brownish-red, central portion, the medulla, and a surrounding outer portion, the cortex.

The Adrenal Cortex of the Mouse. Similar to man, the adrenal glands of the mouse are compound organs derived by union of two originally separate types of tissue. Because of this topographical relationship to one another, these tissues are referred to as cortical and medullary.

Waring[3] has studied the development of the adrenal gland of the mouse. The earliest cortical anlagen found was in an embryo of 12 days. It was distinguished from the surrounding mesenchyme solely by the closer aggregation of its nuclei and its more densely crowded cytoplasmic granules.

---

* This work has been aided by grants to the Roscoe B. Jackson Memorial Laboratory from the Commonwealth Fund, The Anna Fuller Fund, The Jane Coffin Childs Fund for Medical Research, and The National Advisory Cancer Council.

The nuclei were essentially like those of the surrounding mesenchyme, being slightly ovoid and distinctly vacuolated. The anlage of the gonad was well developed at this stage. The fact that there was no connection whatever at this stage between the adrenal anlage and any other organ makes it extremely probable that the anlage had arisen *in situ* by proliferation from the peritoneum. By the end of the 13th day this anlage, which was destined to form the cortex of the adrenal, was consolidated and separated from the general mesenchyme by a sheath.

On the 13th day of foetal life there was a definite sympathetic mass closely applied to the cortical anlage. From it, sympathochromaffin elements eventually migrate into the cortical anlage to form the medulla of the adult adrenal. This sympathetic mass is very quickly developed, for in sections of 12-day-embryos no trace of it was found, while, at 13 days, it was well established. By the day of birth, the sympathochromaffin elements were concentrated at the center of the gland and were beginning to metamorphose into medullary cells. The undifferentiated eosinophil cortical tissue now formed a layer immediately outside the medulla. The lighter eosinophil staining tissue began to show the first distinct signs of the arrangement characteristic of the adult glomerulosa and fasciculata zones. After birth, in both sexes, the juxta-medullary zone increases, eventually increasing more in the female than in the male. This is known as the X zone. The three zones of the adult cortex are distinguishable in both sexes by 25 days: the zona glomerulosa, zona fasciculata, and the zona reticularis. At 35 days (exact time depending on strain), degeneration of the X zone of the male is well established. In the female, the X zone is now at about its maximum development. Degeneration of the X zone in the female is usually complete when the animal is between 100 and 300 days of age, depending upon the strain observed. The X zone of the mouse is probably homologous with the boundary zone of man and of the cat. In the mouse, it has been demonstrated that the X zone increases in width by cell division and not by change in appearance of cells in the inner layers of the permanent cortex. Using the colchicine technique, we have observed that, in the young, adrenal mitoses were evenly distributed throughout the zona fasciculata and the zona glomerulosa. Lack of frequent mitoses at the capsule indicated the absence of cell migration in the normal cortex at this age.

In certain strains of mice, particularly following gonadectomy, the development of the adult adrenal cortex is abnormal. Using the technique of gonadectomy, it has been found that much can be learned about the development of these abnormalities (tumors) of the adrenal cortex.

Nodular Hyperplasia of the Adrenal Cortex. The changes in the adrenal cortex of strain *JAX dba*, which has been studied extensively[1, 4, 5, 6] undergoes pronounced modification following gonadectomy. In all of the gonadectomized strain *dba* animals killed after 3 to 4 months of age, the adrenals have shown characteristic changes which progressively lead to the formation of nodular hyperplasia. Most of the glands were studied in serial section. The earliest changes consisted of the appearance of groups of small, densely arranged cells immediately below the capsule. Eventually these spread between cells of the zona glomerulosa, zona fasciculata, and hypertrophied cells soon to be described. These subcapsular cells are polyhedral in shape, having deeply staining, round nuclei and slightly basophilic cytoplasm. As they extended in between the cell columns of the zona fasciculata they became spindle-shaped and strikingly resembled cells of the ovarian stroma. As the subcapsular cells increased in number by mitoses, they were seen first to interrupt the normal arrangement of the cells of the zona fasciculata and later to invade and replace the cells of all three zones of the cortex. This invasion soon resulted in the formation of a wedge shaped abnormal area. After the apex of the wedge reached the medulla, the sinusoid circulation of this part was disturbed and large, blood-filled lakes were often found surrounding the abnormal areas.

Simultaneously with the appearance of the subcapsular cells, cells which had undergone hypertrophy were found. These have been termed type A and type B cells, respectively. Normally, cells of the zona glomerulosa were small, with relatively large compact nuclei and slightly basophilic cytoplasm. Their cell outlines were indistinct. After undergoing hypertrophy, the nuclei became large and vesicular. The cytoplasm increased in amount, accumulated lipoid, stained lighter, and the cell outlines were more distinct. Such cells were occasionally found in the process of cell division. Later, these cells formed cell nests which were surrounded by spindle-shaped cells.

By increase in size and number of cell components, the wedge-shaped areas changed into round nodules and often involved large parts of the cortex. The structure of such nodules resembled those lutein cells of the mouse ovaries which develop from atretic follicles. The cytoplasm of these cells often contained yellow pigment. Occasionally, the pigmented cells fused and formed large multinuclear giant cells.

In some cases, the enlarging abnormal nodules caused bulging of the surface. In other cases, the nodules projected toward the center of the gland and pushed the medulla into an eccentric position. In several glands, enlargements occurred in both directions. Still further growth resulted in the invasion and finally in the breaking-through of the capsule, after which, a mushroom like outgrowth occurred and resulted in the involvement of the surrounding adipose tissue. In extreme cases, large areas of surrounding tissues were involved. Since circulation was seriously disturbed, necroses, thrombosis, and calcification in the central area have been seen to occur. The same adrenal frequently contained several abnormal nodules which showed different phases of the changes described. Even in the most extreme involvement, some normal cortical and medullary tissue was always present. The advanced changes were visible in the gross, enlarging the gland and giving it a nodular outline.

The same nodular hyperplasia occurred in gonadectomized male and female mice, although at any age period the changes tended to be more extensive and more advanced in the females than in the males.

Adrenal Cortical Carcinoma. In another strain studied extensively following gonadectomy,[7, 8, 10, 11, 12] strain *JAX ce*, other interesting tumors, adrenal cortical carcinomas, have been found. Preliminary changes toward these tumors are very similar to the early changes toward nodular hyperplasia described for strain *JAX dba* and will not be described here. Adrenal cortical carcinomas first appeared in 100 per cent of the ovariectomized females at 6 months of age and were present in 100 per cent of the females in succeeding age groups. None were observed in the intact females of this strain examined. The same tumors occurred with a high frequency in gonadectomized males and not in intact males. As with nodular hyperplasia, the tumors of the male tended to develop later, and with a lower frequency, than in females.

In general, the smaller carcinomas were dense, cellular nodules composed of atypical cells showing frequent mitotic figures. They were imbedded in the outer region of the cortex, in areas already disorganized with changes toward nodular hyperplasia. Expansion into the medulla was evident in all cases as the nodules enlarged. Eventually the medulla and the remaining non-carcinomatous cortical tissue was spread out more or less as a sheet a few cells thick, first over much of the tumor and later, because of the large size of the tumor, over but part of the outer surface. The growths were fairly rounded in their earlier stages, but, as they attained a size of 1.5 cm. or more in diameter, protuberances often developed. The rounded contour was also modified by the adjacent kidney. Not only did the kidney modify the tumor, but the tumor often modified the shape of the kidney. That is, in some instances, the kidney was flattened on its anterior surface; in others, partially covered by the tumor. Invasion of the kidney was not noted, despite this close spatial relationship. Invasion of the liver and metastases to the lung have been observed.

The tumors were made up predominately of two types of tissue. Type I had large polyhedral cells with large oval nuclei containing chromatin granules of medium size. These cells were diffusely arranged. This tissue was not encapsulated, although the surrounding cell columns and sinusoids, which were compressed by the expanding grow that some places, gave this impression. Type II had small cells, cuboidal in shape, and arranged in rows. The cells had large, dark-staining, oval nuclei with evenly distributed coarse chromatin granules. A slight amount of slightly basophilic cytoplasm surrounded each nucleus (hematoxylin and eosin stain). The cells resembled the follicular cells of young ovarian follicles.

Accessory Reproductive Organs. The strain differences are further emphasized by observations made on accessory reproductive organs[9, 10, 11, 12] in the gonadectomized mice.

In strain C57 black, for example, little stimulation of the accessory reproductive organs occurs in the gonadectomized mice in later life. In strain *dba*, the stimulation is pronounced and is as if an estrogen was present. In strain *ce*, the stimulation is as if both estrogens and androgens were present: in some cases predominately like estrogen stimulation and in others predominately like androgen stimulation. In the case of strain *ce*, the stimulation is reproduced when the tumor is transplanted. Since transplantation data is for the most part unpublished, some of the observations will be presented here in considerable detail.

Tumor Transplantation. Within the series of gonadectomized *ce* strain mice with primary adrenal tumors, there were a number of variations in the nature of the accessory reproductive organ development. The present report concerns transplantation studies with 6 adrenal tumors from several of these different types of mice. The results indicate the necessity of evaluating adrenal cortical carcinomas individually, even in an inbred strain such as *ce.* They also indicate that considerable dependability exists for these tumors following transplantation.

All mice in this study were *ce* strain mice. Castration and ovariectomy was performed within 1 to 3 days after birth. Intact females were all virgin mice. The scale for tumor-size classification is the same as that previously used: microscopic to 0.5 cm. in diameter (small); 0.5 cm. to 1.5 cm. in diameter (medium); greater than 1.5 cm. in diameter (large). Since data on the primary tumors and their hosts have in some instances been presented in earlier reports, animal numbers and reference numbers are included for comparison of transplant with primary tumors. Tumor transplantation was accomplished by first cutting the tumor into small pieces with scissors on a piece of sterile glass. A few pieces of the tumor were then transplanted subcutaneously with the aid of a 13 gauge trocar. Both macroscopic and microscopic observations were made on the transplanted tumors and on many organs of the host mice. For histological study, tissues were fixed in a mixture of alcohol, formalin, and acetic acid,[8] embedded in paraffin, sectioned at 8μ, and stained with hematoxylin and eosin.

1. The successful transplantation of one strain *ce* adrenal cortical tumor has been reported.[13] It was transplanted into four classes of animals: (1) gonadectomized females; (2) gonadectomized males; (3) intact females; and (4) intact males. The primary tumor originated in a gonadectomized female 17 months of age. The original tumor and the transplant tumors were associated with an influence which was interpreted as predominately androgenic on the basis of accessory reproductive organ observations.

2. A second adrenal cortical tumor originated in a gonadectomized male which was autopsied at 16 months of age. The primary adrenal tumor was of large size and the histological appearance was that of a typical *ce* strain adrenal cortical carcinoma. This tumor was of particular interest for transplantation because the original host had extensive squamous metaplasia of the prostate epithelium, indicating the presence of estrogenic hormones. Not all gonadectomized male mice with adrenal tumors had this metaplasia. The submaxillary gland was of male type, indicating the presence of androgenic hormones. The mammary glands were not uniformly developed from gland to gland; the most extensive were nearly as well developed as those of a *ce* strain virgin female one year of age. No end buds or alveoli were present.

The tumor was transplanted subcutaneously into a gonadectomized female when the host mouse was 37 days of age. This was the first transplant of this tumor. Autopsy was performed at 209 days, that is, after the transplant tumor had been present for 172 days. The tumor was then of large size. At autopsy the submaxillary gland was of male type. The clitoridean glands were $\frac{1}{4}$ the size of preputial glands in intact males of similar age. The vaginal epithelium was 4 to 5 cell layers thick. The outer layers stained lightly and were infiltrated with leucocytes. Leucocytes were also present in the lumen. The uterus was large in diameter, similar to a virgin female. There were well-developed uterine glands. The epithelial cells lining the lumen were low columnar and closely packed. The nuclei were oval and at the base of the cells. The mammary glands were similar to those of a virgin *ce* strain female of the same age. No nodules, so-called precancerous lesions, were present in the mammary glands of this mouse or any other mouse included in this report. The adrenal glands were without primary tumors. Foci of type A cells were present at the outer edge of the cortex. The cytoplasm of the cortical cells immediately below the type A cells stained lightly. Type B cells were not observed. On the basis of previous observations, extensive areas of type B cells, as well as small primary adrenal cortical tumors, would have been expected in an ovrariectomized *ce* strain mouse of this age. Observations on the accessory sex organs are not incompatible with the interpretation that the tumor transplant produced internal secretions very similar to those produced by the original tumor.

The tumor was also transplanted subcutaneously into a gonadectomized male *via* the above ovariectomized female. The host mouse was 37 days of age at transplantation. Autopsy was performed when the mouse was 294 days of age. The tumor was of medium size at autopsy. The seminal vesicles were small and secretions were not present. There was extensive squamous metaplasia of the prostatic epithelium, as in the mouse with the primary tumor. The lumen of the prostate contained cornified epithelial cells and leucocytes. The submaxillary gland was intermediate between male type and female type, closer to female type than to male type considering the age of the animal. The mammary glands were not uniformly developed from gland to gland. However, all glands except L4 (numbering cephalad to caudad) were developed much more than those of an intact male, and were of medium length and narrow when compared to a strain *ce* virgin female of similar age. There were many small duct branches. No end buds or alveoli were present. One adrenal gland was without tumor. Type A and a few type B cells were present. The other adrenal possessed a tumor of the cortex which, at greatest diameter, occupied an area of about $\frac{1}{3}$ the total width of the gland. Although no record of rate of growth of the tumor transplant was made, the fact that it was only of medium size after 257 days indicates that the growth had been slow. It might be interpreted that the small size of the transplant for several months permitted the occurrence of type B cells and of a primary adrenal cortical carcinoma. Internal secretions of a sex hormone nature in the primary tumor seemed to be similar to that in both transplant tumors, in that in each instance, there seemed to be a pronounced estrogenic effect.

3. Adrenal tumor P2528 originated in a gonadectomized male.[12] The mouse was autopsied at 21 months of age, at which time there were two adrenal tumors, the smaller on the right side. All transplants were of the tumor on the right side. The seminal vesicles were enlarged and secretion copious. The prostates were well developed. Alveolar development of the mammary gland was extensive. The alveoli contained secretions. The submaxillary gland was male type. The anterior lobe of the pituitary was greatly enlarged.

Tumor P2528 was transplanted subcutaneously into a gonadectomized male when the host mouse was 34 days of age. Autopsy was performed when the animal was 327 days of age. The transplant tumor was large at this time (25 mm. in diameter). Grossly, it was observed that the seminal vesicles were $\frac{2}{3}$ normal size. The prostates were well enlarged. The adrenal glands appeared to be normal. The submaxillary gland was large and light in color, similar to that of an intact male mouse. The preputial glands were $\frac{1}{3}$ normal size. The pituitary was normal. The mammary glands were rudimentary. The histological appearance of the tumor transplant was the same as the original tumor. The microscopic appearance of the submaxillary gland, seminal vesicles, prostates, and preputial glands was similar to that in intact strain *ce* males. Small foci of type A cells were present at the outer edge of the cortex in each adrenal. No primary adrenal tumors were present.

Adrenal tumor P2528 was also transplanted subcutaneously into a gonadectomized female when the host mouse was 34 days of age. Autopsy was performed when the animal was 326 days of age. The transplant tumor was of large size. The uterus was medium large, but not well developed. The lumen was not extensively branched. The epithelial lining of the vagina was two cell layers thick. The clitoridean glands were almost the size of the preputial glands of an intact male. The submaxillary gland was male type. The tubular development was greater than is normally seen in *ce* strain male mice and could be identified in the unsectioned gland much as the tubules of a testis can often be seen through the tunica albuginea. The mammary glands were of medium length (compared to a virgin female), narrow, and with a few short branches. There were no end buds or alveoli. Type B cells were not present in the adrenal glands. No primary adrenal tumors were present.

4. Adrenal tumor P2511 originated in a gonadectomized female autopsied at 18 months of age.[10] At autopsy the mouse had two adrenal tumors. Parts of the larger (left) tumor were transplanted. In the original mouse, the submaxillary glands were male type. The uterus was large in diameter, with branched lumen and cystic uterine glands. The mammary glands were moderately developed; a few alveoli were present. The clitoris was unusually large in this female.

Adrenal tumor P2511 was transplanted subcutaneously in a gonadectomized female when the host mouse was 38 days of age. The tumor grew slowly, not being noticeable two months after transplantation. The mouse was autopsied at 376 days of age, at which time the transplant tumor was of medium size. The vaginal epithelium of the host was two cell layers thick, as in an immature mouse. The uterus was medium size in diameter. The submaxillary gland was male type. The clitoridean glands were about $\frac{1}{2}$ as large as the preputial glands of a male. The clitoris was not unusually enlarged. The mammary glands were of moderate length, narrow, and with many small branches. No end buds or alveoli were present. The right adrenal had a tumor of the cortex 2 mm. in diameter. The left adrenal had type A and type B cells but no adrenal tumor.

Adrenal tumor P2511 was also transplanted subcutaneously in a gonadectomized male when the host mouse was 38 days of age. At 373 days of age autopsy was performed. The transplant tumor was large at this time. At autopsy, the seminal vesicles were $\frac{3}{4}$ the size of those in an intact male of similar age. The preputial glands were $\frac{2}{3}$ normal size. The submaxillary gland was male type. The mammary glands were rudimentary. Type A and type B cells were present in each adrenal. No primary adrenal tumors were found.

5. Adrenal tumor P2214 originated in a gonadectomized female mouse.[8, 9] When the mouse was autopsied at 12 months of age, there was a medium-sized adrenal tumor on the left side. The right adrenal was without tumor. The epithelial layer lining the vagina was 12 to 14 cell layers thick. In addition, there was a cornified layer. The uterus was medium in diameter. The epithelial cells lining the lumen were tall columnar. Although the submaxillary gland was male type, its development in this direction was not pronounced. The mammary gland ducts were long and narrow. No end buds or alveoli were present.

Adrenal tumor P2214 was transplanted subcutaneously into an intact male P2565, when the mouse was 79 days of age. Autopsy was performed at 443 days, when the transplant tumor was of medium size. Grossly, it was observed that the seminal vesicles were slightly smaller than expected in a *ce* strain male of this age. Histologically, the seminal vesicles, prostates, and testes were normal. The mammary glands were rudimentary. The submaxillary gland was male type. No primary tumors or foci of type B cells were present in the adrenal glands.

Adrenal tumor P2214 was transplanted subcutaneously in gonadectomized female P2694 via P2565, when the mouse was 42 days of age. The mouse was autopsied when 22 months of age, when the transplant tumor was of large size.

At autopsy the uterus was medium large in diameter. The lumen was well branched. The epithelial cells lining the lumen were low columnar. The vaginal epithelium was two cell layers thick. The submaxillary gland was male type. The mammary glands were similar to those of a *ce* strain virgin female.

6. Adrenal tumor P2182 originated in an ovariectomized female which was autopsied when 10 months of age.[8, 9] At this time, there were two primary adrenal cortical carcinomas forming a tumor mass medium in size.

In the original mouse, the submaxillary glands were intermediate between male and female in type. The vaginal epithelium was 11–12 cell layers thick with a *stratum corneum* in addition. The basal layer of cells were dark staining. There were many cornified cells and a few luecocytes in the lumen. The uterus was of medium size with well-developed uterine glands. Closely packed, tall columnar cells lined the lumen. The cytoplasm of these cells was turbid appearing. A few mitotic figures were present. The blood supply of the uterus was very good. The thyroid had many small follicles. The epithelial cells lining the follicles were cuboidal. The follicles were not all completely filled with secretion. The mammary gland ducts were long, and end buds were present, indicating rapid growth. No alveolar development was observed.

Adrenal tumor P2182 was transplanted into 29 intact *ce* strain mice over 4 transplant generations. It grew successfully in 11 of 20 male mice and in none of 9 female mice. Tumor tissue and other tissue was saved for histological examination from male mice in the first 4 transplant generations. At first, the transplant was almost entirely of type II tumor tissue, a type which had previously been observed to grow with difficulty in competition with type I. In the first and second generation transplants, areas with less cell polarity and with lighter staining cells appeared within the tumor and seemed to be developing into a prominent part of the tumor. In later generations, this tissue was the extensive type.

The testes and accessory reproductive organs of the male mice with tumor transplants were not greatly modified by the tumor transplant. The adrenal glands had wide bands or areas of lipochrome cells between the medulla and cortex.

Pituitary Relationships. There is some evidence that the pituitary has a relationship to these adrenal cortical tumors. Although the problem needs much more study, we have observed that pituitary tumors (anterior lobe abnormalities) may occur in association with the adrenal cortical hyperplasias and carcinomas. These are being investigated in studies now in progress. These pituitary abnormalities did not occur in the control animals. Some evidence has been presented that these pituitary changes are correlated with extensive alveolar development of the mammary glands.[10] Furthermore, in one transplanted adrenal tumor (P2528), where there was an association between pituitary abnormality, adrenal cortical carcinoma, and good alveolar development of the mammary glands, it was observed that, in the mice with transplants of this adrenal tumor, the accessory reproductive organs were well stimulated but the mice did not have either abnormal pituitaries or extensive alveolar development in the mammary glands.

Prevention of Tumors. The prevention of nodular hyperplasia of the adrenal cortex and of adrenal cortical carcinoma is being investigated. The most extensive data comes from subcutaneous implantation of pellets of diethylstilbestrol in gonadectomized strain *ce* male and female mice.[14] In this study, fusion pellets of 25 per cent diethylstilbestrol in cholesterol, average weight 4.8 mg., were used. These were introduced into the subcutaneous tissue of the right axilla when the mice were approximately 7 weeks of age. Only one pellet was used at this age in each mouse and none were implanted later in life. Experimental mice were prepared by removing the gonads of strain *ce* mice immediately after birth. It will be recalled that adrenal cortical carcinomas had previously been observed in 100 per cent of gonadectomized female mice of this strain 6 months of age and older. In gonadectomized male mice, these tumors had occurred starting at 7 months. Diethylstilbestrol-treated mice of both sexes observed up to 14 months of age did not have these tumors. Although data are not available for protection at extremely advanced ages, the results are encouraging for continued protection, since nodular hyperphasia of the adrenal cortex, which preceded the occurrence of carcinomas in previous experiments, was also prevented by the use of diethylstilbestrol pellets. Further study needs to be made to find protective agents for these tumors which will not at the same time cause other serious body disturbances. In addition to undesirable histological changes in the accessory reproductive organs, pituitary tumors of the type caused by estrogenic hormones were observed in the mice as early as 7 months of age.

Genetic and Endocrine Factors. There are important genetic factors back of the occurrence of adrenal cortical tumors. This is indicated by pronounced strain differences in response to gonadectomy. The adrenal cortex of strain *JAX C57 brown* or *JAX C57 black*, for example, undergoes only slight change in size or structure following gonadectomy. Strain *JAX dba* develops nodular hyperplasia of the adrenal cortex and strain *JAX ce*, adrenal cortical carcinoma. Hybridization experiments now in progress also point to the importance of the genetic factors.

Endocrine factors are also of great influence. In our series, adrenal cortical tumors of the types described in this report did not occur without gonadectomy. Following gonadectomy, certain endocrine preparations will prevent their occurrence in a genetically susceptible strain. It is known that there is an intimate relationship between the adrenal cortex and the pituitary. In these first experiments, the relationship is evidenced by occasional abnormalities of the anterior lobe of the pituitary in the experimental and not in the control series.

An hypothesis might be that there is: (a) an increased inactivation (utilization) of adrenal cortical hormone following gonadectomy; (b) the fall in cortical hormone level (or possibly only the fall in gonadal hormone level) removed an inhibitory influence on the adrenocorticotropic activity of the anterior lobe of the pituitary; and (c) increased adrenocorticotropic activity led to hypertrophy of the adrenal cortex. Cortical carcinoma occurrence of the type described is the result of certain genetic factors, together with a certain balance of the endocrine factors.

It is hoped that eventually the problem will be reduced to: how the adrenal cortical carcinoma originates from responsible factors.

References

1. Fekete, E., George W. Woolley, & C. C. Little. 1941. Histological changes following ovariectomy in mice. I. *dba* high tumor strain. J. Exp. Med. **74:** 1–8.
2. Fekete, E. & C. C. Little. 1945. Histological study of adrenal cortical tumors of gonadectomized mice. Cancer Research **5:** 220–226.
3. Waring, H. 1945. The development of the adrenal gland of the mouse. Quart. J. Micr. Sci. **78:** 329–367.
4. Woolley, George W., E. Fekete, & C. C. Little. 1939. Mammary tumor development in mice ovariectomized at birth. Proc. Nat. Acad. Sci. **25:** 277–279.
5. Woolley, George W., E. Fekete, & C. C. Little. 1941. Effect of castration in the dilute brown strain of mice. Endocrinology **28:** 341–343.

6. Woolley, George W., E. Fekete, & C. C. Little. 1940. Differences between high and low breast tumor strains of mice when ovariectomized at birth. Proc. Soc. Exp. Biol. & Med. **45:** 796–798.
7. Woolley, George W., E. Fekete, & C. C. Little. 1943. Gonadectomy and adrenal neoplasms. Science **97:** 291.
8. Woolley, George W. & C. C. Little. 1945. The incidence of adrenal cortical carcinoma in gonadectomized female mice of the extreme dilution strain. I. Observations on the adrenal cortex. Cancer Res. **5:** 193–202.
9. Woolley, George W. & C. C. Little. 1945. The incidence of adrenal cortical carcinoma in gonadectomized female mice of the extreme dilution strain. II. Observations on the accessory sex organs. Cancer Res. **5:** 203–210.
10. Woolley, George W. & C. C. Little. 1945. The incidence of adrenal cortical carcinoma in gonadectomized female mice in the extreme dilution strain. III. Observations on the adrenal glands and accessory sex organs of mice 12 to 24 months of age. Cancer Res. **5:** 321–327.
11. Woolley, George W. & C. C. Little. 1945. The incidence of adrenal cortical carcinoma in gondaectomized male mice of the extreme dilution strain. Cancer Res. **5:** 211–219.
12. Woolley, George W. & C. C. Little. 1945. The incidence of adrenal cortical carcinoma in male mice of the extreme dilution strain over one year of age. Cancer Res. **5:** 506–509.
13. Woolley, George W. & C. C. Little. 1947. Transplantation of an adrenal cortical carcinoma. Cancer Res. **6:** 712–717.
14. Woolley, George W. & C. C. Little. 1946. Prevention of adrenal cortical carcinoma by diethylstilbestrol. Proc. Nat. Acad. Sci. **32:** 239–240.

URINARY EXCRETION OF ADRENAL CORTICAL STEROIDS

By Eleanor H. Venning and J. S. L. Browne
McGill University Clinic, Royal Victoria Hospital, Montreal, Canada

It has become apparent in recent years, through the work of many investigators, that the adrenal cortex has a variety of functions. The substances so far derived from it may be roughly divided into three groups: (1) compounds which act mainly on electrolyte metabolism; (2) compounds which affect protein and carbohydrate metabolism, and (3) compounds which have properties of the sex hormones. Into the last group fall the estrogens, progesterone, and the androgens. The latter also affect protein metabolism.

Metabolites of these various adrenal hormones are excreted in the urine and an attempt has been made to correlate these urinary substances with their possible precursors in the gland or with definite types of adrenal function. In man, two groups of urinary steroids have been associated with adrenal metabolism, namely, the 17-ketosteroids and the glycogenic corticoids.

The first group, the 17-ketosteroids, are characterized by the facts that they have a carbonyl group at C-17 and give a typical color reaction with alkaline dinitrobenzene. They have been associated with the adrenal, because they are found to be increased in cases of adrenal tumor and decreased in cases of hypofunction of the adrenal. In the male, the 17-ketosteroids are derived from both the adrenal and the testis, whereas, in the female, they are entirely of adrenal origin. The adrenal precursors of these substances have not yet been identified, but they are considered to be associated with the androgenic function of the adrenal, and to have an anabolic effect on body protein.

The glycogenic corticoids differ from the 17-ketosteroids in that they possess the same biological activity as the corticosterones, *i.e.*, they are capable of prolonging the life of adrenalectomized animals and will cause an increase in liver glycogen when administered to fasting adrenalectomized animals. They are readily extractable from urine with chloroform or ethylene dichloride, are strongly reducing substances, and are heat labile. They are associated with that group of adrenal substances which are concerned with carbohydrate metabolism. .

The urinary corticoids have been assayed by the method of Venning, Kasmin, and Bell.[1] It is a bioassay based upon the ability of these hormones to cause a deposition of glycogen in the livers of adrenalectomized fasted mice. Briefly, the assay is carried out in the following manner: The urinary extract is prepared by acidifying a 48-hour specimen of urine to pH 1 and extracting it with ethylene dichloride or chloroform. After distilling off the solvent, the residue is taken up in chloroform and the solution is extracted with N/10 alkali and water. The chloroform is evaporated off and the residue obtained is dissolved in a small amount of alcohol and is finally made up into an extract containing 10 per cent alcohol and 5 per cent glucose. This extract is injected into mice which have been adrenalectomized 4 days previously and fasted for 16 hours prior to the assay. The extract is administered in 7 divided doses over a period of 5½ hours, and, 1 hour later, the livers are removed and are analysed for glycogen. The amount of glycogen deposited is compared with amounts laid down by 17-hydroxy 11-dehydrocorticosterone. One glycogenic unit is equivalent to the activity of 1 microgram of this compound. The administration of small amounts of glucose greatly sensitized the method, so that adrenal cortical steroids in the order of 8–10 micrograms can be detected. Thus, the measurement of the urinary corticoids offers a means of assessing a type of adrenal function different from that associated with the excretion of 17-ketosteroids. In the following study, the variations in the excretion of these two groups of adrenal metabolites have been investigated in normal individuals and in patients with various endocrine disorders. The effect of stress and trauma, such as severe muscular exercise, infections, burns, and fractures, has also been studied.

Excretion in the Normal Individual (FIGURE 1).[2] In 14 normal adult women, aged from 21 to 48 years, urinary corticoid activity ranged from 25 to 65 glycogenic units per 24 hours, with an average value of 41 units. In 14 normal adult men, between the ages of 20 to 51 years, the corticoids varied from 40 to 85 glycogenic units per 24 hours, with an average of 60 units. In the females, the 17-ketosteroids ranged from 6–18 mg./24 hours with an average value of 11.6 mg., while, in the males, they ranged from 10–25 mg./24 hours, with an average value of 16.6 mg. In old age, the values for both these fractions are decreased.

The urine from eight new-born male infants was collected during the first four days of life and an amount of urine equivalent to a 12-hour specimen was injected per mouse. In four of these cases, no activity could be detected; in four, amounts in the order of 10–15 units were found. By the ages of 2½ and 3 years, 35 to 40 glycogenic units were present in the urine and at 5½ years, adult male values were present. The 17-ketosteroids were low in the younger children, increased in value with advancing age, and reached adult values later than the corticoids.

Some variation is seen in the excretion of these substances when followed from day to day in the same individual, both in males and females, but values remain within the normal range established by single assays in a number of different individuals. Similar variations are observed in the excretion of 17-ketosteroids. The values for 17-ketosteroids and for glycogenic corticoids do not necessarily parallel each other even in the adults. No correlation could be found with the rhythmic excretion of the sex hormones in the menstrual cycle.

Excretion in Endocrine Disorders (FIGURE 2).[3] Our studies were extended to examine the excretion of these two groups of compounds in various endocrine and other disorders:

(1) *Addison's Disease:* Four cases were studied, one male and three females, all showing typical symptoms and signs of this disease. In all four

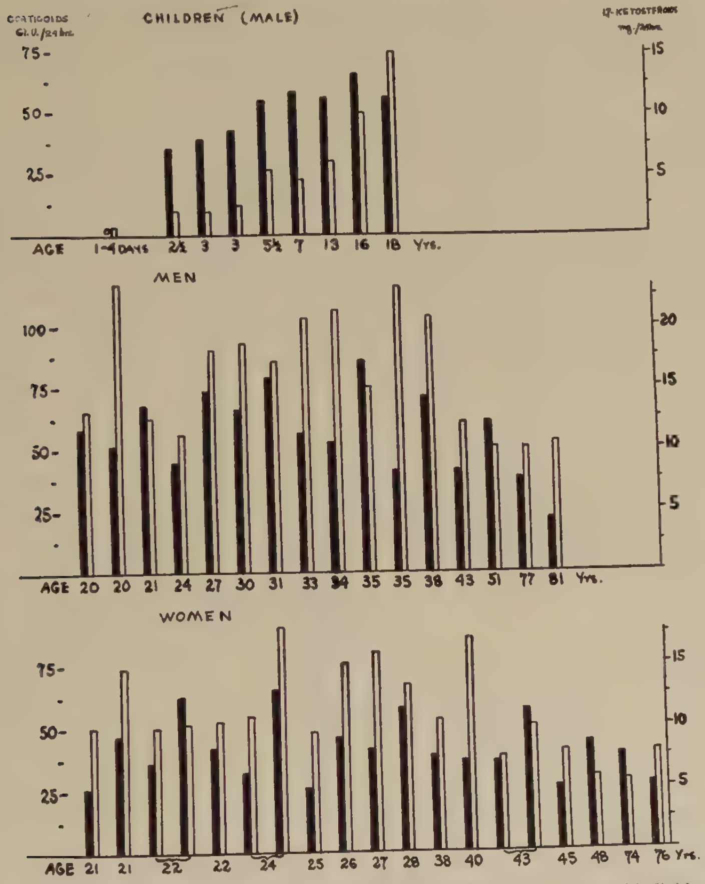


FIGURE 1. Excretion of glycogenic corticoids and 17-ketosteroids in a group of normal individuals. The black columns represent the daily excretion of corticoids in terms of glycogenic units; the white columns, mg. of 17-ketosteroids.

cases, the 17-ketosteroids were low. In three of the cases, less than the amount of glycogenic corticoid activity detectable by the method was present. In the fourth case, however, amounts varied from below normal to low normal on various occasions, suggesting the possibility that one type of function of the adrenal may decrease more rapidly than another, or independently of the other functions.

(2) *Panhypopituitarism:* Five cases were studied, three males and two females. All showed many signs of pituitary hypofunction. Both the 17-ketosteroids and the glycogenic corticoids were very low. It is of interest that these individuals did not show marked evidence of electrolyte disturbances in spite of the similarity of their glycogenic corticoid excretion to that found in Addison's disease. This may be further evidence for the possibility of dissociation between two types of adrenal hypofunction.

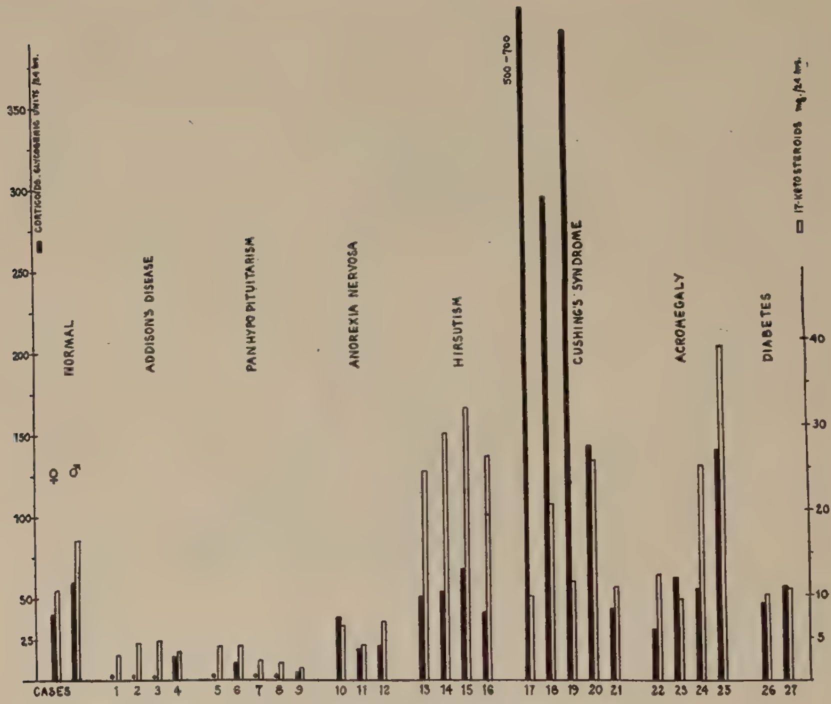


FIGURE 2. Excretion of glycogenic corticoids and 17-ketosteroids in various endocrine disorders.

(3) *Anorexia Nervosa:* Three cases which are typical as regards history and physical findings were studied. In these cases, the pituitary hypofunction has been regarded as occurring as a consequence of the malnutrition. The 17-ketosteroids were low, but in general not so low as those found in the cases of panhypopituitarism, described in the previous section. Glycogenic corticoids were present in all three cases, but in amounts lower than normal.

(4) *Hirsutism (Simple):* In four cases of simple hirsutism, the glycogenic corticoids were normal, while the 17-ketosteroids were definitely raised, ranging from 25 to 35 mg./24 hrs. None of these patients showed any other signs of virilism except the increase in body and facial hair. Only in Case 16 were the adrenals explored and, in this case, the right adrenal was slightly enlarged.

(5) *Cushing's Syndrome:* In all the cases of active Cushing's syndrome studied, the values for glycogenic corticoids were markedly raised, reaching values of 700 units in two cases. The 17-ketosteroids were either normal or slightly elevated. In certain cases where the process had been arrested or had become inactive, normal values were found, even though the patient still showed clinically the same evidences of Cushing's syndrome (Case 21). The degree of impairment of the glucose tolerance curve did not always parallel the increase in corticoid excretion.

(6) *Acromegaly:* In one untreated case of acromegaly of recent origin,

elevated glycogenic corticoids and 17-ketosteroids were found. In three other cases of long standing duration, in whom the pituitary had been irradiated, normal values for both corticoids and 17-ketosteroids were present.

(7) *Diabetes:* Several cases of diabetes have been studied and all have shown normal values. Three cases which were insulin-resistant did not have elevated glycogenic corticoids.

In general, it may be said that the determination of urinary glycogenic corticoids, in addition to the 17-ketosteroids, is of assistance in elucidating various types of cases whose signs and symptoms suggest abnormality of adrenal function. The two determinations frequently do not parallel each other, thus suggesting that the two types of adrenal function associated with these metabolites may be independent of one another.

Other Factors Influencing the Excretion of Glycogenic Corticoids.

(1) *Muscular Exercise:* It was found that strenuous muscular exercise caused a marked increase in output of these adrenal substances. On two separate occasions, pooled urine was obtained from a group of 14 healthy army recruits before and after a 4 mile route march. The glycogenic corticoids were increased from 46 to 160 glycogenic units per liter in the first experiment, and from 60 to 210 units in the second one. The 17-ketosteroids were not determinded.

(2) *Effect of Trauma*[4]*:* During the past three years, in collaboration with metabolic studies on protein metabolism, an extensive investigation has been carried out on the excretion of glycogenic corticoids following acute trauma, such as surgical operations, burns, fractures, wounds, and infections, in both the previously healthy individual and the chronically debilitated person. The results may be summed up very briefly as follows: When a healthy well-nourished individual is subjected to an acute trauma, there is an immediate and rapid increase in output of the glycogenic corticoids, reaching a maximum in most cases within 3 to 4 days. This elevated output is maintained for a varying period of time, depending to some extent upon the severity of the damage inflicted. In most cases, the corticoid excretion has returned to the normal level by the end of the third week, but, in several cases, the increased excretion of corticoids is maintained for a much longer period. In some of the cases studied, values around 400 units have been found at the peak of the excretion, although generally, the values lie between 200 and 300 units. There is usually an immediate rise in 17-ketosteroids, but this increase is not always maintained and the changing levels of 17-ketosteroids and corticoids do not necessarily parallel one another.

In FIGURE 3 is shown the rapid increase in excretion of glycogenic corticoids and 17-ketosteroids in a well-nourished individual following fractures of the humerus and femur. On the 12th day after injury, a bone plating to the left femur was done. This second trauma was followed by a secondary rise in corticoid excretion. By the 15th day, the corticoids were back to normal and remained at this level throughout convalescence.

In the chronically ill person suffering from malnutrition, the response of

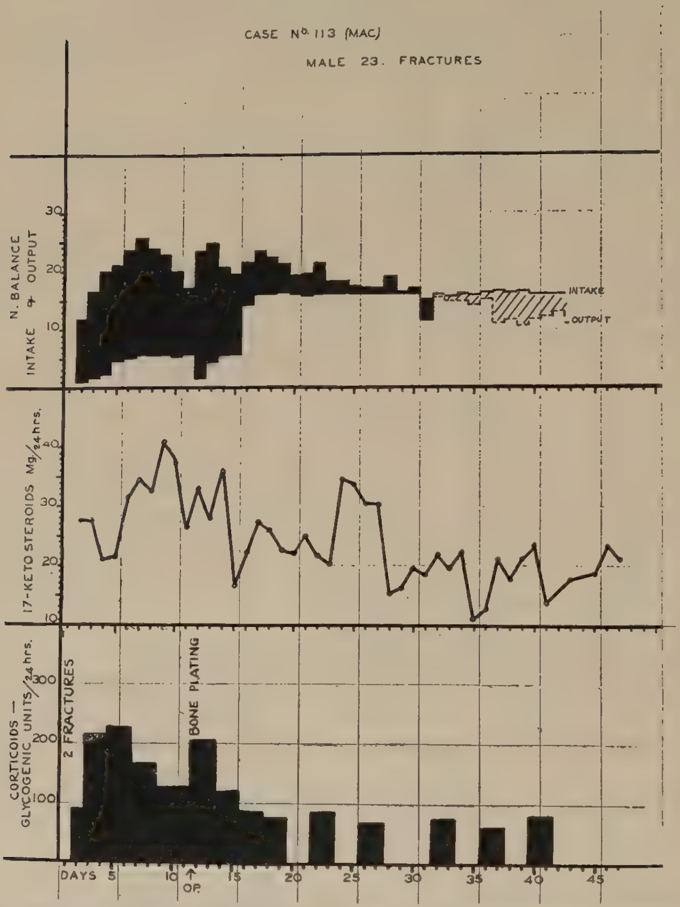


FIGURE 3. The excretion of glycogenic corticoids, 17-ketosteroids, and nitrogen balance in a well-nourished individual following fractures.

the adrenal to trauma is markedly different from that seen in the healthy individual. In a large percentage of the cases studied, the excretion of corticoids in these patients is above the normal limits. Following operation, there may be no increase in output of glycogenic corticoids, or else, the increase is maintained for only a few days, reaching normal levels again by the end of the first week.

Excretion in Pregnancy.[5] Earlier investigators had noted by direct observation that the adrenal gland undergoes definite hypertrophy during pregnancy. Nine cases in all were studied. In general, the curves obtained for the excretion of corticoids and 17-ketosteroids followed the same pattern, although some of the patients excreted larger amounts of metabolites than others. During the first trimester, all showed an increased excretion of corticoids. After this initial rise, the excretion returned to normal levels, then, between the 140th and the 160th days, began to rise again, reaching values over 300 units in some cases. There was usually a falling-off in output before parturition. Within a few days after delivery, the urinary corticoids were back to normal level again. In FIGURE 4 are shown the excretion rates of various hormones, including the corticoids, in a case of normal pregnancy.

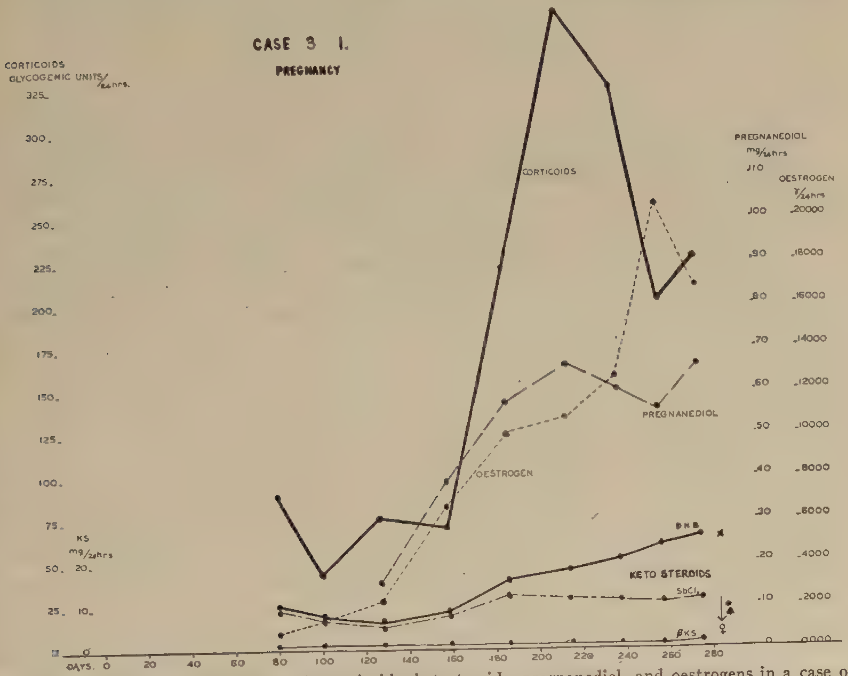


FIGURE 4. Excretion of glycogenic corticoids, ketosteroids, pregnanediol, and oestrogens in a case of normal pregnancy. The arrow denotes the time of delivery; X = corticoid excretion after delivery.

The ketosteroids were followed by two colorimetric determinations: (a) color reaction with alkaline dinitrobenzene (this method includes both 17- and 20-ketosteroids); (b) color reaction with antimony trichloride (this method is more specific for 17-ketosteroids). It was found that with the reagent antimony trichloride no increase of 17-ketosteroids occurred, while with alkaline dinitrobenzene there was an increase in color index beginning between the 140th to 160th day. This increase in ketosteroids as measured by the Zimmerman reaction is attributed to the increased excretion of 20-ketosteroids.

Summary

The excretion of urinary corticoids and 17-ketosteroids has been studied in a series of normal individuals and in endocrine disorders and other pathological cases.

These two groups of urinary metabolites represent different adrenal functions, and, from their excretion rates under various condition, it is suggested that these functions may vary independently of one another.

Strenuous exercise and acute trauma cause an increase in the output of the glycogenic corticoids. The well-nourished individual shows a more marked response than the malnourished one to damage.

In pregnancy, there is a rise in urinary glycogenic corticoids, but no parallel increase in 17-ketosteroids.

Bibliography

1. Venning, Eleanor H., V. Kazmin, & J. C Bell. 1946. Biological assay of adrenal corticoids. Endocrinology **38:** 79.
2. Venning, Eleanor H. & V. Kazmin. 1946. Excretion of urinary corticoids and 17-ketosteroids in the normal individual. Endocrinology **39:** 131.
3. Venning, Eleanor H. & J. S. L. Browne. 1947. Excretion of glycogenic corticoids and 17-ketosteroids in various endocrine and other disorders. J. Clin. Endocrin **7:** 79.
4. Venning, Eleanor H. & J. S. L. Browne. 1944. Excretion of 'cortin like" material following damage: 117. Conference on Metabolic Aspects of Convalescence. Josiah Macy Jr. Foundation.
5. Venning, Eleanor H. 1946. Adrenal Function in pregnancy. Endocrinology **39:** 203.

ADRENAL CORTEX FUNCTION IN STRESS

By GREGORY PINCUS[*]

The Worcester Foundation for Experimental Biology, the Memorial Foundation for Neuro-Endocrine Research, and the Department of Psychiatry of the Massachusetts General Hospital, Boston, Massachusetts

Introduction: Animals and men respond to a great variety of damages and stresses with prompt secretion from the adrenal cortex.[1-3] We have found that this typical adrenal-cortex response to short, acute stress is deficient in psychotic men generally.[3-6]

The ordinary concept of the stress response is based on studies of the alarm reaction in which (a) the stress stimulus activated the anterior pituitary to secrete adrenocorticotrophic hormone and (b) the adrenal cortex, stimulated by the adrenocorticotrophin, released corticosteroid that (c) induces certain target organ activity involving such phenomena as lymphatic tissue breakdown, potassium release from muscle, glycogen deposition in liver, and so on. In this paper, we shall present data on men and animals which are pertinent to each of these three phases of the alarm reaction sequence. Our ultimate objective is to understand the significance of the disturbance of this mechanism in mental disease.

In this paper, we should like, first of all, to present what evidence we have concerning the degree of dependence of certain stress-response indices upon the adrenal cortex in animals and men. Secondly, we present data on the behavior of these indices in psychotic men under stress. Finally, we have data on psychoneurotic subjects to compare with those assembled for normal and for psychotic subjects.

Indices of Adrenal Cortex Secretion. We have previously reported data on two measures of adrenal cortex secretion in man: the 17-ketosteroid excretion and the number of circulating blood lymphocytes.[3-6] The latter can also be used as an index of the secretion of 11-oxygenated corticosteroids[7,8] in animals. In this paper we shall present data on other biochemical variables that reflect aspects of adrenal cortex function. These are: (a) the neutral reducing lipids of urine, which represent in large part the corticosteroids possessing a sugar-like (reducing) side chain[9,10]; (b) urinary uric acid, which represents the purine metabolite excreted in greatly increased amount after adrenal cortex hormone administration[11]; and (c) urinary potassium and sodium, which alter notably in amount after stress,[1] and the excretion of which is governed in large part by certain adrenal cortex steroids.[12]

Our objective in employing these various indices of adrenal cortex secretion is to see if different stresses affect any of them differentially in either normal or psychopathic subjects.

---

[*] Research Professor of Physiology, Tufts Medical College, Boston, Mass.

These researches were conducted in collaboration with Drs. H. Hoagland, H. Freeman, J. Finesinger, A. Watkins, M. Brazier, E. Shantz, Messrs. F. Elmadjian, J. Carlo, D. Stone, and Mrs. L. Romanoff. Grants-in-aid were received from the National Research Council Committee on Endocrinology, the Baruch Committee on Physical Medicine, and G. D. Searle and Co. The Upjohn Company kindly supplied the adrenal cortex extract used.

Glucose and the Alarm Reaction. The lymphocytopenia following stress and the administration of 11-oxocorticosteroids may be rather faithfully simulated by the administration of glucose to both rats[13] and men.[14] FIGURE 1 presents data on normal and adrenalectomized rats subjected to one-dose and two-dose sugar tolerance tests. The fact that the adrenalectomized rats show no significant lymphocytopenia during the test, whereas the normal rats do, indicates that the blood lymphocyte changes are mediated by the adrenals. It is notable that there is a significant negative correlation between the lymphocyte and the blood sugar changes during the tolerance test in the normal rats, whereas no such correlation is found in the adrenalectomized animals (FIGURE 2).

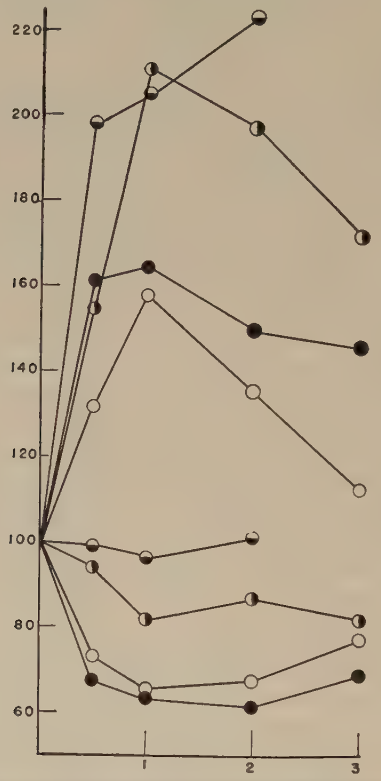


FIGURE 1. The mean percentage changes in blood sugar and lymphocytes following the administration of dextrose to rats. Abscissa: time in hours. Ordinate: per cent. ○ = normal rats, one dose test; ● = normal rats, two dose test; ◑ = adrenalectomized rats, one dose test; ◒ = adrenalectomized rats, two dose test. The blood sugar values are in the upper portion of the figure, denoting increases in all groups; the lymphocyte values are in the lower portion of the figure. (From Endocrinology **39**, 1946)

Freeman and Elmadjian[14] have shown that a lymphocytopenia occurs in normal men in the two-dose (Exton-Rose) sugar tolerance test. Through the kindness of Drs. Fuller Albright and E. B. Astwood, we have been able to conduct Exton-Rose glucose tolerance tests with three Addisonian patients. In addition to the blood sugar and lymphocyte values, we have

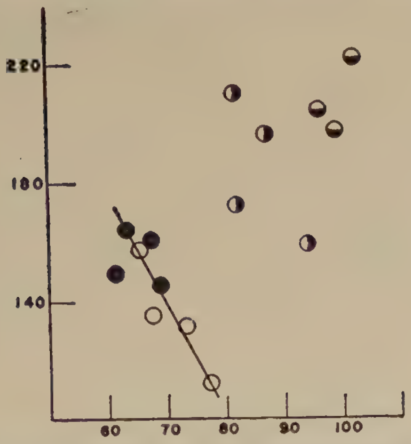


FIGURE 2. The mean percentage changes in blood sugar are plotted against the percentage changes in lymphocytes. Abscissa: lymphocyte percentages. Ordinate: blood sugar percentages. Symbols as in FIGURE 1. Note that the data for the normal rats indicate that rising blood sugar is accompanied by relative lymphocytopenia, and that this does not hold with the data for adrenalectomized rats. (From Endocrinology **39**, 1946)

determined neutral urinary reducing lipid and urinary uric acid in these subjects. A comparison of the data obtained with them and on a group of five normal men examined at the same time is presented in FIGURE 3. The normal men exhibit the characteristic sugar tolerance curve with an accompanying blood lymphocyte decline during the first hour. The Addisonian patients show a distinctly abnormal tolerance curve with some decline in lymphocytes which is, however, proportionately much less than that observed in the normal subjects. The latter show a significant 17 per cent decline at the end of the hour's test, whereas the patients exhibit a 7 per cent drop which is not statistically significant. The normal subjects respond to the administration of sugar by excreting increased amounts of neutral reducing lipid, whereas the characteristic extremely low level of these substances in the patients' urines is scarcely affected. Although neither group show any significant urinary uric acid changes, the extremely low level of output in the Addison's cases is noteworthy.

The foregoing is taken to demonstrate that, in both rats and men, the administration of glucose leads to the increased production by the adrenal glands of corticosteroid hormone.

A Comparison of the Effects on Man of Glucose Administration and Pursuit-Meter Stress. We have previously presented evidence that the stresses of heat and pursuit-meter operation lead to ketosteroiduria and lymphocytopenia in normal men.[3-6] Using other possible indices of adrenocortical function, we have compared the effects of the Exton-Rose glucose tolerance test and the operation of the Hoagland-Werthessen pursuit-meter. FIGURE 4 presents the data thus far obtained with eight normal men who were given a sugar tolerance test and who, one week later, operated the pursuit-meter. Both tests were taken under basal conditions. The blood and urine data of FIGURE 4 are presented as percentages of the pre-stress values.

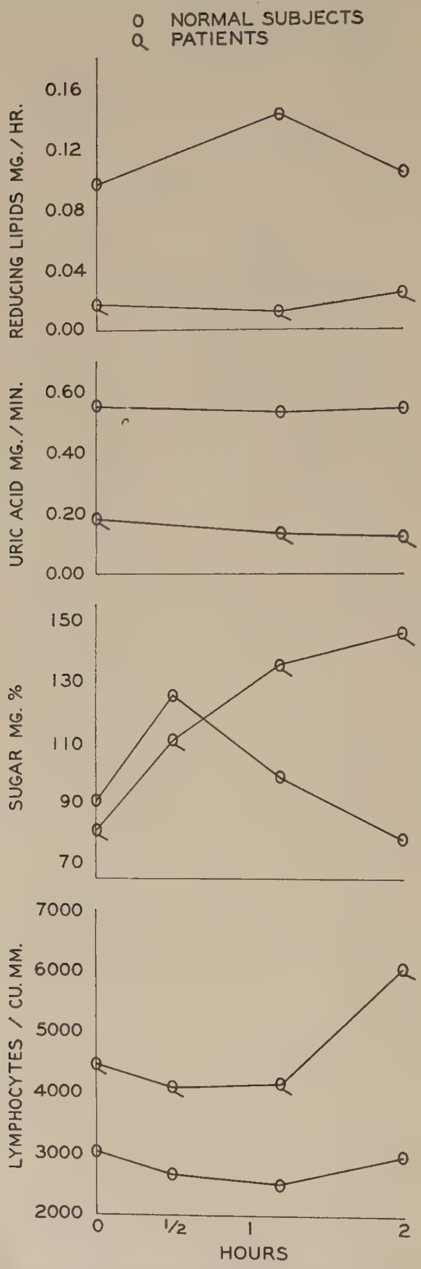


FIGURE 3. Mean variations in blood lymphocyte counts in blood sugar, urinary uric acid, and neutral reducing lipids in 5 normal subjects and three Addisonian patients in the course of Exton-Rose sugar tolerance tests.

The significant features of these data are: (a) both sugar administration and pursuit-meter operation are accompanied by significant increases in

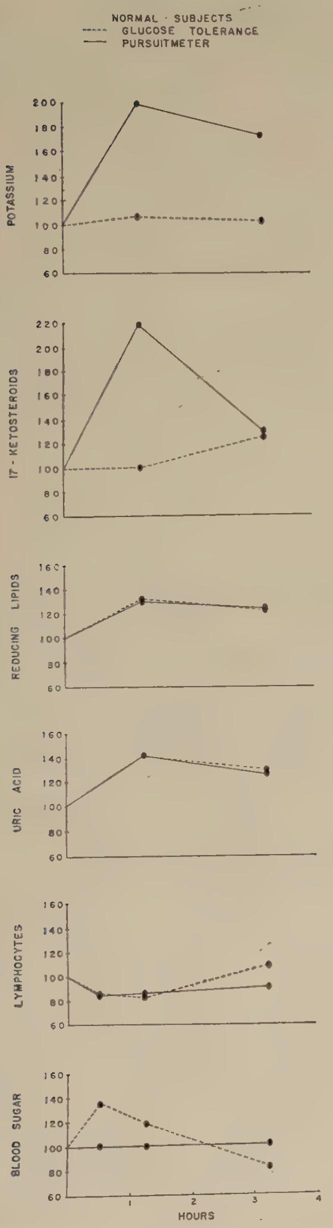


FIGURE 4

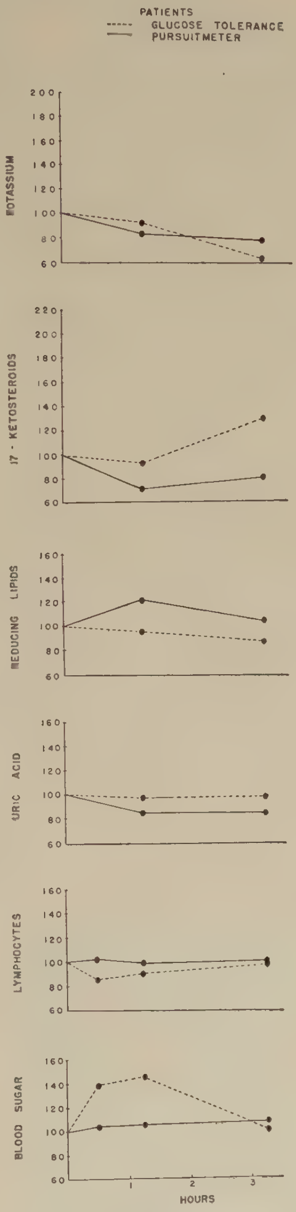


FIGURE 5

FIGURE 4 (Left-hand column). Mean variations in blood sugar and lymphocytes and in urinary potassium, 17-ketosteroids, neutral reducing lipids, and uric acid in eight non-psychotic men during and after glucose administration (- - - -) and pursuit-meter operation (——). Each test lasted for one hour.

FIGURE 5 (Right-hand column). Data as in FIGURE 4, but for fifteen psychotic men.

neutral reducing lipid outputs; (b) the urinary uric acid increases significantly after the sugar administration and after pursuit-meter stress; (c) the 17-ketosteroid output shows the characteristic rise, with a subsequent drop, previously described for pursuit-meter operation,[3] whereas, after sugar administration, the 17-ketosteroid output rises to some extent in the post-stress period; (d) the initial extent of blood lymphocyte decline is about the same after both the sugar and pursuit-meter tests, but there is a continued low level after pursuit-meter operation not observed in the sugar tolerance data; (e) the mean blood sugar change accompanying pursuit-meter operation is insignificant and is not at all correlated with the lymphocyte changes; and (f) there is a marked sustained increase of potassium excretion following pursuit-meter operation, whereas sugar administration leads to only slight change.

These data are consistent with the notion that sugar administration evokes the secretion of sugar-active corticosteroids, whereas pursuit-meter operation evokes a secretion of electrolyte-controlling hormone and 17-ketosteroid precursors as well. In fact, there is even the suggestion that the 17-ketosteroid precursor production is inhibited by sugar administration.

Glucose and Stress in Psychotic Men. We have data on fifteen schizophrenic men subjected in alternate weeks to the glucose tolerance and pursuit-meter tests. The mean data for these subjects are presented in FIGURE 5. It is clear that these men, on the average, do not exhibit the sort of responses shown by the non-psychotic men: (a) the neutral urinary reducing lipids show no increase in response to sugar and a small rise after pursuit-meter stress; (b) the uric acid output fails to show any increase in either situation; (c) the 17-ketosteroid output declines in both tests; (d) there is no notable lymphocytopenia in either test, although a large mean change in blood sugar is shown in the tolerance test and a small average increase occurs in the pursuit-meter data; and (e) after sugar administration and pursuit-meter stress, there is a continued decline in potassium excretion.

These data are consistent with the notion that the responsivity to either stimulative test of the adrenal cortex in the psychotic men is diminished or absent. Our original intention was to discover if there were any indications of differential reactivity in the psychotic subjects. The present data are inadequate for a detailed survey. However, Freeman and Elmadjian[15] have found that psychotic men given an Exton-Rose sugar tolerance test may be divided into two (nearly equal) groups: (a) those who show an inverse relationship between blood sugar and blood lymphocyte concentration (i.e., the relationship observed in normal men); and (b) those who show no inverse relationship, usually with no change in lymphocyte concentration. Actually in the data here presented, a similar division of individuals may be made, but there is no consistent correlated change in the other measures of adrenocortical function.

It may be concluded that the psychotic men who fail to show lymphocytopenia after sugar or pursuit-meter stress have an unreactive lymphatic system. That this is not so is shown by data on such men receiving adrenal cortex hormone. In FIGURE 6, are presented data on such subjects and a similar group of normal controls. It is clear that the psychotic men receiving adrenal cortex extract-in-oil react with a lymphocytopenia as extensive as that of the normal men receiving the same dosage.

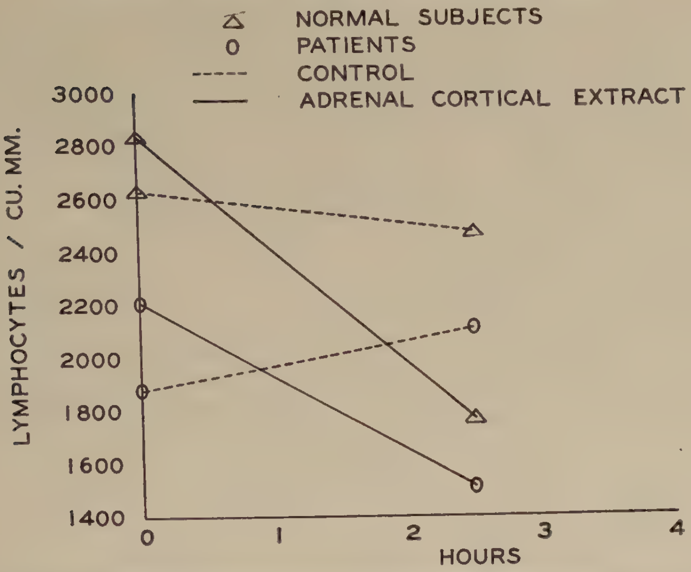


FIGURE 6. Five normal subjects and seven psychotic men received no medication on a control day (- - -) and 10 cc. of Lipo-adrenal extract (Upjohn) on an experimental day (——). Note that the lymphocytopenia on the experimental day is approximately the same in both groups of subjects.

Adrenocortical Function in Psychoneurotic Subjects. We have previously demonstrated that there is a diurnal rhythm of blood lymphocyte concentration in normal men and women.[16] Since this is a fairly regular increase from waking onward, and the obverse of the 17-ketosteroid rhythm,[17] we concluded that the adjustment to waking life involved, initially, a large secretory drain on the adrenal cortex followed by declining secretory activity through the day. Psychotic men generally appeared to show a flatter diurnal rhythm, i.e., less change of activity with waking and through the day.

We have similar data on the changes in blood lymphocytes occurring in psychoneurotic men and women. In FIGURE 7, are presented the mean curves relating lymphocyte number to time of day in these subjects compared with similar curves for normal and psychotic subjects. These graphs demonstrate: (a) that the absolute lymphocyte counts tend to be higher in psychotic subjects than in normal subjects, whereas in psychoneurotic subjects they tend to be lower, especially in the women with hysteria; (b) that the rate of change in lymphocyte number in psychoneurotic subjects is markedly reduced compared with normals. The latter finding is further amplified in TABLE 1, where the mean percentage change in lymphocyte number per hour is given for various groups of subjects. By calculating percentage changes, we obviate differences in absolute levels which are characteristic for each individual. It may be seen that the normal hourly rate of diurnal increase in blood lymphocytes is decreased by about 20 per cent in the male anxiety cases, by 48 per cent in the female anxiety cases and by 73 per cent in the hysterias. Statistically significant decline is had only for the hysterias, but the implication is that the stresses of daily life evoke frequent adrenal responses in the psychoneurotic subjects. They

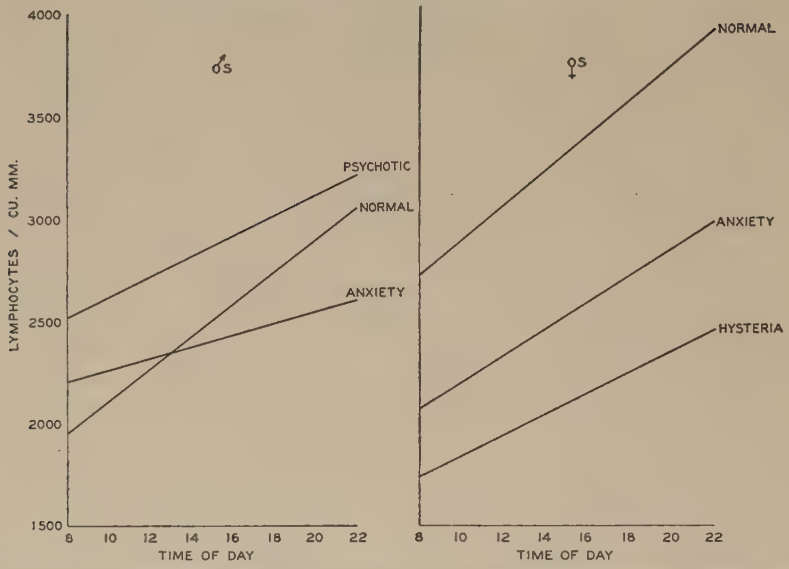


FIGURE 7. The regression of lymphocyte number on time of day for men and women of various mental types.

TABLE 1

MEAN PERCENTAGE CHANGE IN CIRCULATING LYMPHOCYTES FROM WAKING TO SLEEP IN VARIOUS GROUPS OF SUBJECTS

Type of subjects	Sex	Number subjects	Number of daily observa-tions	Mean % change in lymphocyte number per hr.	Coefficient of variation %
Normal	♂	13	51	2.26 ±0.485	148.2
Anxiety neurosis	♂	9	48	1.80 ±0.518	199.4
Normal	♀	13	40	2.57 ±0.615	175.9
Anxiety neurosis	♀	6	24	1.35 ±0.923	334.8
Hysteria	♀	8	39	0.70 ±0.719	641.4

tend not to let the adrenals rest. Furthermore, the high values of the coefficients of variation in the psychoneurotic groups indicate large fluctuation in the circulating lymphocytes.

We have a limited amount of data on the effect of certain stressful procedures on the circulating lymphocytes of psychoneurotic subjects. These data are presented in TABLE 2. The male patients were anxiety neurosis cases, the female patients chiefly hysteria cases. Again, the lymphocyte changes in the male patients are not significantly different from those in the control subjects, but, at one and three hours, the female patients have significant sharp drops in blood lymphocyte concentration. They appear to over-react to the stress.

Five of the psychoneurotic women were interviewed for one hour by a psychiatrist and indifferent matter discussed. On a succeeding day, an interview involving considerable probing and psychological stress was had.

TABLE 2

MEAN PERCENTAGE CHANGE IN CIRCULATING LYMPHOCYTES OF VARIOUS SUBJECTS BREATHING AIR CONTAINING 9 PER CENT O_2 FOR 30 MINUTES

(Room air was breathed through a mask for the first 30 minutes, 9 per cent O_2 introduced for the next 30 minutes, and the mask removed at the end of the hour.)

Type of subject	Sex	Number	Mean % decrease in lymphocytes @		
			½ hour	1 hour	3 hours
Normal	♂	5	15.5	11.4	13.0
Psychoneurotic	♂	6	16.1	19.9	8.1
Psychoneurotic	♀	5	0.4	28.8	35.1

The blood lymphocyte changes occurring are presented in FIGURE 8. It is quite clear that neither immediately after nor some time after the non-

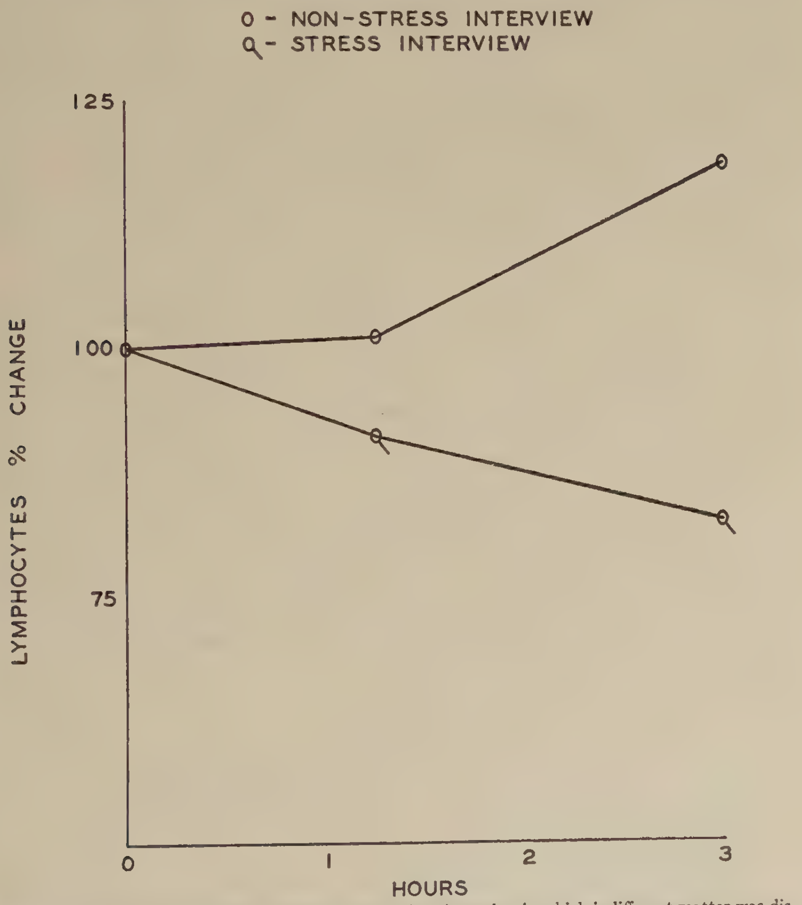


FIGURE 8. The effects on blood lymphocyte number of an interview in which indifferent matter was discussed (non-stress) and of an interview designed to create mental tension (stress). Data on five psychoneurotic women.

stress interview did any lymphocyte decrease occur, whereas progressive lymphocyte fall followed the stressful interview.

Discussion. The foregoing data demonstrate that means for measuring adrenal cortex responses in man are readily available. Furthermore, it is possible to differentiate responses evoked by different stimuli. Thus, in normal men, glucose administration evokes an increase in neutral reducing lipids in the urine but a decrease of 17-ketosteroids, whereas pursuit-meter operation evokes an increase of both. Increased potassium excretion and lymphocytopenia follow the pursuit-meter stress, but only the lympnocytopenia follows the sugar tolerance test.

By applying these tests to psychotic men, we obtain unequivocal evidence of a marked damping or absence of adrenal cortex response. The point of origin of this block in the alarm reaction is not evident from these data. It seems probable that the hormone target organs not are refractory, since patients respond like normal subjects to adrenal cortex extract administration. That the hormone-secreting mechanism of the adrenal cortex is at fault does not seem likely, since (a) the psychotic patients give no evidence of Addisonian levels of hormone secretion or excretion and (b) about half of them respond with lymphocytopenia to sugar administration. The responsivity of the adrenal cortex in these psychotic men can be tested by administering adrenocorticotrophin, and, in a few preliminary experiments, completely normal reactivity has been found in some men but no responsivity in others. It is our intention to determine the source of the block by proceeding to examine pituitary reactivity and thence the central nervous controls of the pituitary.

The data on psychoneurotic subjects thus far assembled indicate no particular block in the adrenal cortex activation mechanism. Rather, there are indications of overactivity of the adrenal cortex. This needs to be substantiated by further analytical data. The indications of rather large fluctuations in adrenal cortex activity in these cases implies a very labile reactivity. It may be that the crux of the psychoneurotic stress-responsivity lies in the source of this lability. Is it in the adrenal cortex itself?—in the pituitary?—in the hypothalamic control of the pituitary?

In presenting these beginnings of a systematic analysis of mental disease in terms of hormonal dysfunction, cautious interpretation is mandatory. Gross endocrinopathy is clearly absent, but it is perhaps noteworthy that those hormonal mechanisms involving homeostatic adjustment are the ones that seem to be malfunctional.

References

1. Selye, H. J. 1946. Clin. Endocrinol. **6:** 117.
2. Long, C. N. H. 1947. Recent Progress in Hormone Research **1:** 99.
3. Pincus, G. 1947. Recent Progress in Hormone Research **1:** 123.
4. Freeman, W., G. Pincus, & E. D. Glover. 1944. Endocrinol. **35:** 215.
5. Pincus, G. & F. Elmadjian. 1946. J. Clin. Endocrinol. **6:** 285.
6. Hoagland, H., G. Pincus, & F. Elmadjian. 1946. J. Clin. Endocrinol. **6:** 301.
7. Dougherty, T. F. & A. White. 1944. Endocrinol. **35:** 1.
8. Elmadjian, F. & G. Pincus. 1945. Endocrinol. **37:** 47.
9. Heard, R. D. H., & H. Sobel. 1946. J. Biol. Chem. **165:** 687.

10. HEARD, R. D. H., H. SOBEL, & E. H. VENNING. 1946. J. Biol. Chem. **165:** 699.
11. BABAD, P. 1939. Arch. Int. Physiol. **49:** 327.
12. SELYE, H. 1936. Canadian Med. Assoc. J. **34:** 706.
13. ELMADJIAN, F., H. FREEMAN, & G. PINCUS. 1946. Endocrinol. **39:** 293.
14. FREEMAN, H. & F. ELMADJIAN. 1946. J. Clin. Endocrinol. **6:** 668.
15. FREEMAN, H. & F. ELMADJIAN. 1947. Psychosomatic Med. **9:** 226.
16. ELMADJIAN, F. & G. PINCUS. 1946. J. Clin. Endocrinol. **6:** 287.
17. PINCUS, G. 1943. J. Clin. Endocrinol. **3:** 195.

CLINICAL STUDIES IN ADDISON'S DISEASE*

By GEORGE W. THORN, PETER H. FORSHAM, F. T. GARNET PRUNTY,† GRACE E. BERGNER,‡ AND A. GORMAN HILLS

Department of Medicine, Harvard Medical School, the Medical Clinic, Peter Bent Brigham Hospital, Boston, Massachusetts

General Considerations

In following a series of one hundred and fifty-eight cases of Addison's disease,[1] it is apparent that two outstanding changes have occurred during the past decade:

1. There has been a marked decrease in the proportion of patients with tuberculosis as the etiology of Addison's disease; *i.e.*, tuberculosis is responsible now for adrenal insufficiency in only half the authors' patients.
2. There has been a considerable prolongation of life. Whereas, in the past, 80 per cent of patients with Addison's disease died within two years,[2] 50 per cent of the patients in the authors' series have survived a seven-year period of treatment (FIGURE 1). This remarkable improvement in life expectancy appears to be due in large measure to (a) the extensive use of synthetic desoxycorticosterone acetate,§ particularly as pellets implanted subcutaneously, combined with the use of whole adrenal cortical extract in emergencies when food intake is not adequate, and (b) the decrease in the incidence of tuberculous etiology, with its extremely unfavorable effect on the general management and prognosis of Addison's disease and the always present danger of a recurrence of activity.

With an increase in the period of survival, it is becoming steadily more important to improve the physical and mental status of these patients to a point where they can become self-supporting, or at least not remain a complete burden to society. The availability of 11-oxy-steroids in addition to desoxycorticosterone would appear to be a prerequisite in this endeavor.

Diagnostic Tests

The diagnosis of classical cases of Addison's disease presents little difficulty and may be suspected on the basis of typical signs and symptoms such as those observed in one hundred patients in the authors' series (TABLE 1). In general, Addison's disease should be considered in: (1) patients with unexplained weight loss and asthenia; (2) patients with unexplained gastro-intestinal symptoms, especially in the presence of tuberculosis; and (3) a rare group characterized by manifestations of a neurological disease, including syncopal attacks and seizures occasionally accompanied by focal electroencephalographic changes.

Reduced heart size and hypotension are present in most instances. A normal heart size in an untreated patient with adrenal insufficiency suggests concurrent primary heart disease or hyperthyroidism.

---

* This study was aided, in part, by a grant from the Committee on Research in Endocrinology, National Research Council.

† Rockefeller Travelling Fellow.

‡ Commonwealth Fund Fellow.

§ We are indebted to Dr. Ernst Oppenheimer of Ciba Pharmaceutical Products, Inc., for the Percorten pellets and solution used in these studies.

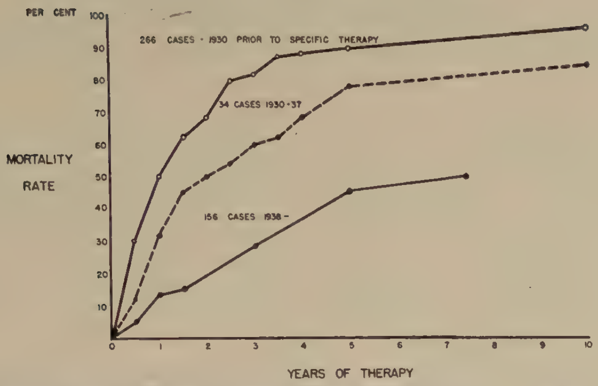


FIGURE 1. Mortality rate in Addison's disease. (From THORN, G. W., P. H. FORSHAM, & K. EMERSON, JR. 1949. American Lecture Series. Charles C. Thomas. Springfield.)

TABLE 1
INCIDENCE OF SIGNS AND SYMPTOMS IN ADDISON'S DISEASE
(100 patients)

Weakness and fatigability	100%
Weight loss	100%
Increased pigmentation	94%
Anorexia, nausea, and vomiting	85%
Diarrhea	21%
Salt-craving	19%
Muscle pain	16%

Hypometabolism (minus 10 to minus 15 per cent) is typical of the disease A basal metabolic rate of zero or above usually indicates complicating hyperthyroidism. The classical signs and symptoms of the latter may appear minimal in spite of a definite enlargement of the thyroid and continued weight loss. Conversely, a basal metabolic rate below minus 25 per cent suggests coexisting hypothyroidism. With gonadal insufficiency, it suggests hypopituitarism as the cause of both adrenal insufficiency and the other deficiencies. This condition is characterized by the paucity of pigmentary abnormalities and a relative preponderance of hypoglycemia as opposed to hypochloremia.

In more advanced cases of Addison's disease, one may expect hemoconcentration and a reduction in serum sodium and chloride level, with a rise in serum potassium. The ratio (normal = 30) of sodium to potassium will be decreased.

Measurement of the twenty-four hour urinary excretion of 17-ketosteroids is a valuable diagnostic aid (TABLE 2). Unfortunately, many non-specific

TABLE 2
URINARY 17-KETOSTEROID EXCRETION (MG. PER DAY) IN 70 PATIENTS WITH ADDISON'S DISEASE

	Range	Mean	Mean Deviation
Males (37)	1.0–8.0	4.2	±2.7
Females (33)	0.1–8.6	3.0	±2.0

Normal means for males, 15.0; for females, 10.0.

conditions may result in a low 17-ketosteroid excretion. This is particularly true of diseases associated with a marked inanition. On the other hand, a normal 17-ketosteroid excretion value is reliable in *ruling out* Addison's disease.

The establishment of milder or earlier degrees of adrenal cortical deficiency may be extremely difficult and will require, in most instances, the use of tests designed to place the adrenal cortex under stress. Such tolerance tests may be applied to most of the known functions of the adrenal cortex. The Robinson-Kepler-Power water test[3] for water diuresis is a perfectly safe screening test, but tends to give a positive response in patients with kidney disease, liver disease, and certain abnormalities of the gastro-intestinal tract, as well as in Addison's disease.

The Cutler-Power-Wilder test for sodium retention[4] is valuable, and a positive response is highly significant, but with the marked dehydration and potassium retention that may ensue there is considerable risk of an adrenal crisis. The test requires careful dietary management throughout and should be employed in a hospital under strict observation.

Tolerance tests based on the derangement in carbohydrate and protein metabolism are designed to reveal an inability to preserve or replenish carbohydrate levels once depressed by various means. The induction of hypoglycemia at the end of a glucose tolerance test (FIGURE 2), after the twentieth hour of a twenty-four hour fast (FIGURE 3), or following the administration of a small dose of insulin, not only aids in the diagnosis, but assists in assessing the need for a high carbohydrate diet with frequent feedings, supplementary whole adrenal extract therapy, or both.

Unfortunately, the application of any of these tests represents a real hazard to the patient. Following the intravenous glucose tolerance test, as well as the administration of excessive amounts of intravenous glucose, we have observed on many occasions a characteristic syndrome conveniently termed "glucose fever." After an essentially uneventful glucose tolerance test, with or without hypoglycemic manifestations, which may occur at blood sugar levels as high as 60 mg. per cent on occasion, all appears well. Some twelve hours later, the patient suddenly experiences a chill which is followed by marked hyperpyrexia (FIGURE 4). Usually, several waves of fever appear, and a normal temperature is reached again after two or three peaks. Malaise, aches and pains, nausea and vomiting, and varying degrees of disorientation accompany these episodes. Although a careful search is made for sources of infection, bacteriological studies are negative, and pyrogens cannot be demonstrated as a rule in the solutions used intravenously. Administration of whole adrenal extract supplemented with plasma or albumin has proved life-saving in treating patients with this complication. The similarity of this reaction to the manifestations commonly associated with water intoxication and the rather dramatic improvement following the administration of human albumin have led the authors to consider this reaction a form of abnormal cellular edema involving the central nervous system.

Recently, the use of pituitary adrenocorticotrophic hormone (ACTH)*

---

* We are indebted to Dr. John R. Mote of the Armour Laboratories for the purified pituitary adrenocorticotrophic hormone used in these studies.

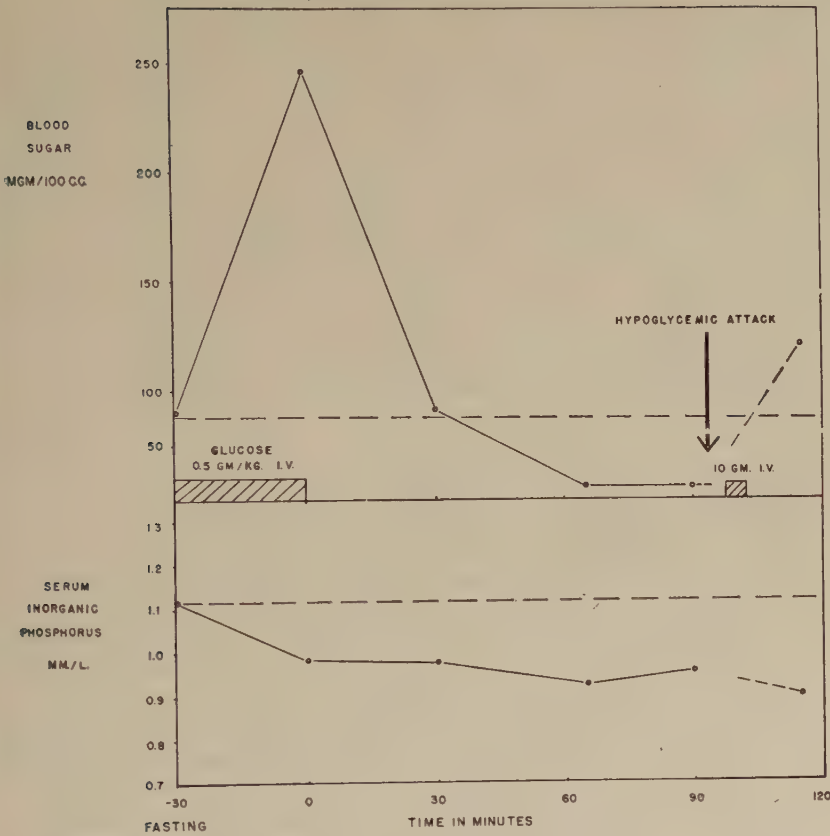


FIGURE 2. Intravenous glucose tolerance curve, Addison's disease. Patient M. N. ♀., age 45.

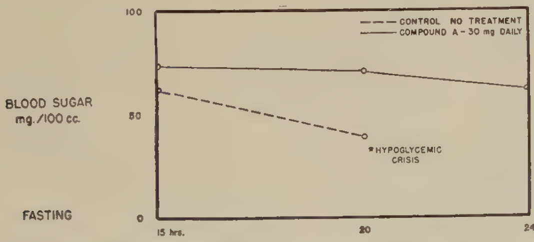


FIGURE 3. Blood sugar during prolonged fast, Addison's disease. Patient V. A. ♀., age 37. (From FORSHAM, P. H., G. W. THORN, G. E. BERGNER, & K. EMERSON, JR. 1946. Am. J. Med. 1: 105.)

has been found to be of considerable aid in testing for the presence of a normal adrenocortical response. Studies on patients with an intact adrenal cortex have shown that many metabolic changes follow the administration of ACTH. There is an increased nitrogen output, increased uric acid excretion, a decreased sodium output, an increased fasting blood sugar level, an increased 17-ketosteroid and 11-oxy-steroid excretion, and a decrease in circulating lymphocytes and eosinophils, with a variable leucocytosis. The

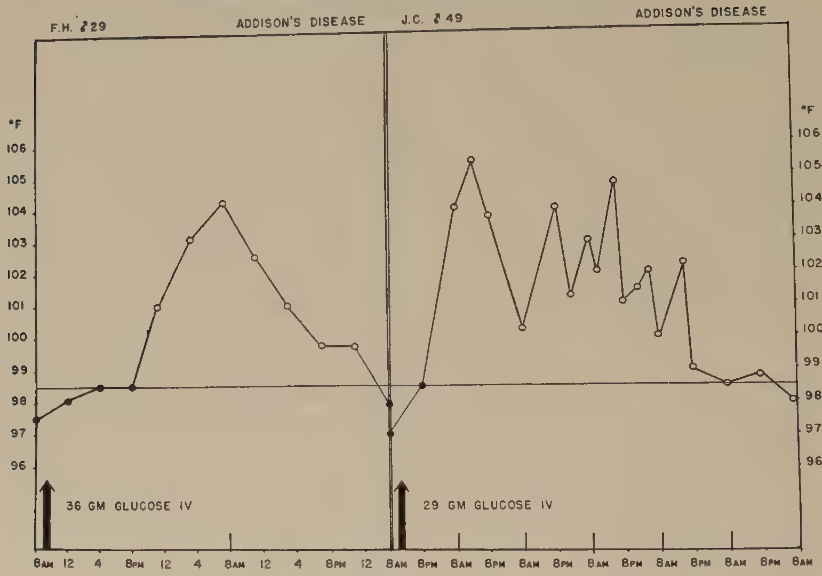


FIGURE 4. Febrile response following IV-glucose administration.

most characteristic and easily measured changes were the increase in the uric acid, conveniently expressed in terms of the urinary uric acid-creatinine ratio, and the decrease in circulating lymphocytes and eosinophils. Direct enumeration of eosinophils was employed, using a Levy counting chamber and an eosin-acetone-water diluting fluid.[5] The average response of patients with Addison's disease to intramuscular ACTH as judged by any of the above changes fell far below that of normal subjects and miscellaneous hospital patients. That this was due to an inability of the adrenal cortex to increase further the output of cortical hormones and not to an abnormality in the various end-organs, was proved by experiments such as that shown in FIGURE 5. In the case of the uric acid-creatinine ratio (FIGURE 6) and the decrease in eosinophils (FIGURE 7), no patient with Addison's disease manifested a response to ACTH as great as that of the least responsive subject with normal adrenal cortical reserve.

On the basis of these findings, a simple clinical test has been devised. The patient is fasted overnight, and urine is collected from 6:00 a.m. to 8:00 a.m. At 8:00 a.m., 25 mg. of ACTH in saline is administered intramuscularly. A second urine collection is made from 9:00 a.m. to 12:00 noon, and then the patient is given lunch. Blood elements are determined at 8:00 a.m. and at 12:00 noon, the uric acid-creatinine ratio is estimated in both urine samples, and the per cent increase from the early to the late specimen is calculated. Renal insufficiency may mask the urinary response. The hematological changes, however, will still show the characteristic difference between the normal subject and patients with primary adrenal cortical insufficiency.

In testing for mild forms of adrenal cortical insufficiency, a therapeutic

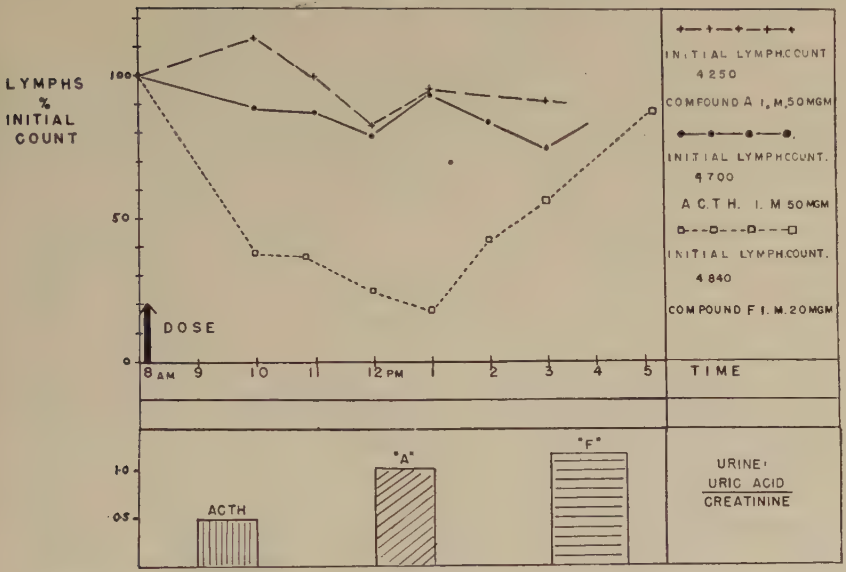


FIGURE 5. Effect of single doses of ACTH and compounds A and F in Addison's disease. Patient C. S. ♀

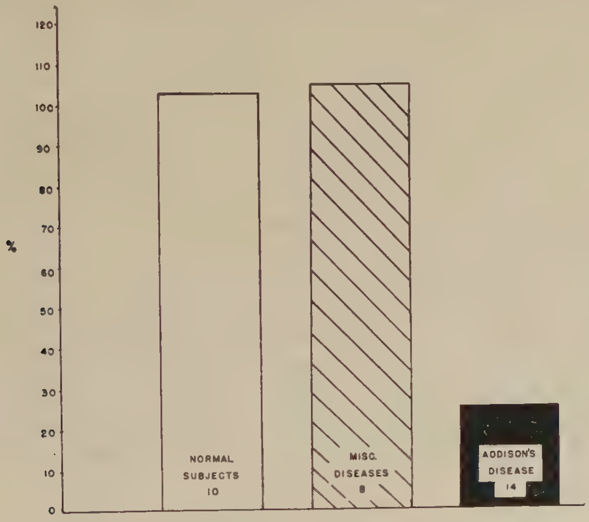


FIGURE 6. Increase in $\frac{\text{uric acid}}{\text{creatinine}}$ ratio following ACTH administration.

trial with desoxycorticosteroue acetate forms a safe and satisfactory method of diagnosis. The test as performed on an ambulatory patient is as follows: Weights taken prior to breakfast are recorded, and daily basal blood pressures are obtained whenever possible. The patient is told not to make any major change in the usual salt or fluid intake. Hematocrit is determined initially and again at the termination of ten days of Percorten therapy, 0.5

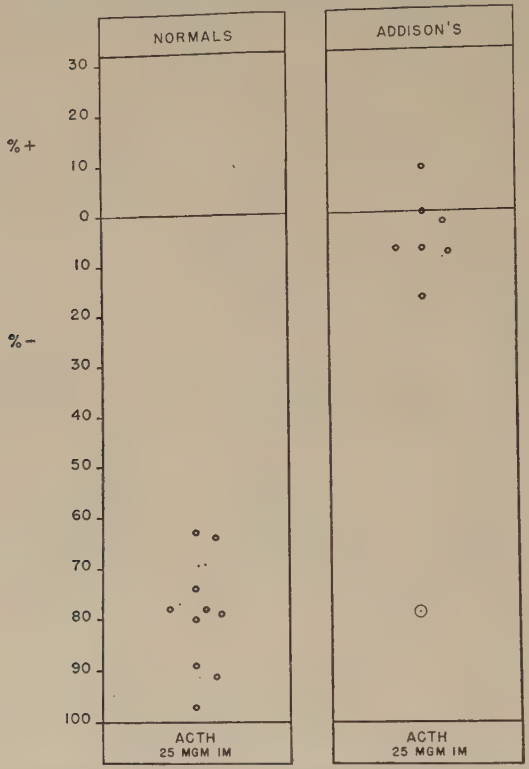


Figure 7. Per cent change of eosinophil count four hours after ACTH. The point ⊙ represents the fall in eosinophils observed in one of the patients with Addison's disease given 20 mg. of Compound F intramuscularly.

cc. (2.5 mg. desoxycorticosterone acetate intramuscularly) daily. Changes in weight, blood pressure, hematocrit, and clinical response are followed. This trial is repeated using 0.5 cc. of sesame oil (placebo) for ten days, and the Percorten is then resumed.* In patients with Addison's disease, there will be clinical improvement, weight gain, hemodilution, and possibly a slight increase in blood pressure under Percorten therapy, with regression upon institution of placebo injections. Neurasthenics will show only insignificant physiological responses and will continue to feel better during the placebo period.

Treatment

The treatment of Addison's disease is an example of substitution therapy which has become more specific over the years. The milestones in the use of hormonal preparations, isolated or synthesized, are presented in TABLE 3.

It is now generally accepted that the following abnormalities exist in adrenal insufficiency:

Decreased sodium and chloride retention.

---

* Ciba Pharmaceutical Products, Inc., provides a diagnostic package which contains both Percorten solution and placebo sesame oil.

TABLE 3

HISTORICAL DEVELOPMENT OF HORMONE THERAPY

1895.	Use of glycerine extract of fresh adrenals by Osler.
1929.	Preparation of active adrenal extracts by Hartmann, Rogoff, and Swingle.
1938.	Partial synthesis of desoxycorticosterone by Reichstein.
1943.	Purified porcine extract in oil with a high 11-oxy-steroid content (Upjohn).
1946.	Partial synthesis of dehydrocorticosterone by Kendall and by Reichstein.

Diminished carbohydrate preservation and synthesis from protein.

Inadequate utilization of fat.

Moderate impairment of androgenic functions.

Decreased lympholysis and possibly a decreased release of gamma globulin and antibodies.

Characteristic pigmentation.

The importance of supplementary sodium chloride therapy in the treatment of Addison's disease, as suggested by Loeb[6] and Harrop,[7] has been substantiated by continued clinical experience and is an effective means of reducing the cost of specific treatment in those patients requiring hormone.

Experience in the use of desoxycorticosterone acetate, with its powerful sodium chloride retaining action, has clearly shown that for patients treated with this synthetic hormone a low potassium diet is neither desirable nor helpful and is, indeed, contra-indicated in most instances. Supplementary sodium chloride is to be used with great caution in conjunction with desoxycorticosterone. By regulating the dosage carefully, it has been possible to avoid many of the undesirable side effects found more frequently in the earlier period of treatment[1] and to improve greatly both the clinical condition and life expectancy of patients with Addison's disease. Over the past nine years, pellets of desoxycorticosterone acetate, implanted subcutaneously, have been used with success in over two hundred patients. This method obviates the necessity for daily injections of hormone and eliminates the possibility of allergic reactions to the oily medium in which the synthetic hormone is dissolved. The average number of pellets implanted (weight 125 mg.) in one hundred of our patients between January, 1944, and September, 1946 has been four for males and three for females, approximately once a year. Although hypertension is frequently reported as a complication to desoxycorticosterone acetate therapy, the mean blood pressure of fifty patients recently admitted to the Peter Bent Brigham Hospital for implantation of pellets has been 114/17 mm. Hg at the time of entry, and 125/81 mm. Hg one to six months after implantation. Heart size, below normal in all uncomplicated cases, has been brought back to, but not above, normal after treatment in fifty cases recently analyzed. Thus, hypertension, edema, and heart failure have been practically eliminated as complications of overdosage with desoxycorticosterone, but the occurrence of arthralgia and tendon contraction is still a debilitating complication which arises in an occasional case. The treatment includes the administration of potassium citrate, a reduction in the sodium chloride intake, and administration of small amounts of prostigmine to release painful contractures.

Over one-half of the authors' patients do well on desoxycorticosterone acetate supplemented by a high caloric diet. Since desoxycorticosterone fails, however, to correct the disturbances in carbohydrate, protein, and fat metabolism which exist in this disease, supplementary whole adrenal extract therapy has been used whenever patients show signs of weakness and hypoglycemia and experience frequent bouts of non-specific infections when on the standard type of therapy. The very high cost makes this type of therapy available to the vast majority of the authors' patients only for periods of inadequate food intake, medical emergencies, and surgical intervention.

Preparations showing carbohydrate regulating activity are shown in TABLE 4. Bovine extract in aqueous solution has a short period of action (four hours) with an early onset and lends itself to use in acute emergencies when at least 50 cc. per day must be given intramuscularly.

TABLE 4

ADRENAL CORTEX PREPARATIONS POSSESSING CARBOHYDRATE REGULATING EFFECT

1. Whole extract (bovine) in aqueous solution.
2. Whole extract (porcine) in oil.
3. Synthetic 11-dehydrocorticosterone (Compound A, Kendall).
4. Naturally occurring 11-oxy steroids (Compounds A, B, E, and F).

The preparation of a purified adrenal extract standardized in terms of crystalline steroid hormone for its carbohydrate regulating activity (Lipo-Adrenal Cortex, Upjohn),* has been a great advance in the treatment of patients with Addison's disease. The longer continued action of this hormone preparation in oil (eight hours) reduces the number of injections per day.

Unfortunately, adequate supplementary extract treatment, either 10 to 20 cc. of adrenal cortex aqueous preparation daily or 2 to 3 cc. of Lipo-Adrenal Cortex daily, raises the cost of treatment to approximately $2500 yearly; whereas desoxycorticosterone acetate treatment alone (pellets implanted subcutaneously or daily intramuscular injectious of hormone in oil) costs approximately $100 yearly.

In crises and in acute emergencies, adrenal cortical extract has proved life-saving in many of the authors' cases. It is important to differentiate between a crisis occurring in a patient under treatment with desoxycorticosterone acetate, and therefore usually well-hydrated, from a crisis occurring in a patient who has never received treatment. In the latter case, the use of large quantities of sodium chloride parenterally is of great benefit; whereas excessive administration of salt and water to a patient on desoxycorticosterone acetate treatment may lead to fatal overhydration. The authors have used large quantities of whole adrenal extract, however, in the treatment of crisis induced by infection or trauma in a patient under desoxycorticosterone acetate therapy. Salt retaining equivalents are presented in TABLE 5. It has also been possible with adequate supplementary extract therapy to carry patients with Addison's disease through major surgical procedures.

Interest and hope are aroused by the recent synthesis of an 11-oxy adrenal

---

* We are indebted to Dr. E. Gifford Upjohn of The Upjohn Company for the Lipo-Adrenal cortex used in these studies.

TABLE 5

APPROXIMATE EQUIVALENTS

Sodium-Chloride Retaining Effect

Preparation	Dose	Administration
Desoxycorticosterone acetate in oil	1 mg.	Once daily I.M.
Pellets of desoxycorticosterone acetate (125 mg.)	2	q 9–12 mos.
Adrenal extract (bovine)*	5 cc.	q 4 hrs. I.M.
Lipo-Adrenal cortex (porcine)*	1 cc.	q 8 hrs. I.M.

* Equivalence in carbohydrate regulating capacity is the same for these preparations.

steroid, Compound A,* according to the method of Kendall. Although it is doubtful that this preparation or its more potent relatives, Compounds E and F, will be available commercially for some period of time, it may be appropriate to record the authors' limited experience with this Compound,[8] which differs in some respects from the findings reported by others.[9]

Fourteen patients with classical Addison's disease have been treated with this preparation. With a daily dose of approximately 30 mg., a small decrease in sodium and chloride excretion was observed in conjunction with a moderate increase in the renal excretion of total nitrogen, inorganic phosphorus, and potassium. Compound A appeared to be approximately one-twentieth as active as desoxycorticosterone acetate in its sodium retaining capacity and less than one-half as active as Compound F in carbohydrate regulating potency. The most outstanding effect which was observed under Compound A therapy was the ability of patients to withstand a twenty-four hour fast which under control conditions resulted in a severe hypoglycemic reaction (FIGURE 3).

In metabolic experiments with Compound A, it was evident that clinical improvement could not be ascribed entirely to glucose derived from protein (increased gluconeogenesis), since the increased quantity of nitrogen excreted was so small. The increase in uric-acid excretion, which was observed in thirteen of the fourteen patients who received Compound A, was a more constant finding than the slight increase in nitrogen excretion observed. There was no detectable fall in circulating lymphocytes.

The addition of Compound A therapy to a basic regimen of desoxycorticosterone acetate resulted in clinical improvement in the four patients with severe Addison's disease to whom it was possible to administer 20 to 30 mg. daily for periods of two to three months. Regression followed rapidly the withdrawal of Compound A.

It is of interest that none of the therapeutic measures employed to date have truly altered the state of pigmentation in the authors' patients beyond a generalized lightening associated with rehydration and an unexplained, slight, but definite decrease in generalized tan upon the long-continued administration of Compound A.

---

* We are indebted to the late Dr. D. F. Robertson of Merck & Co., Inc. for the Compound A used in these studies.

Conclusions

The diagnosis of adrenal cortical insufficiency has been greatly improved over the past decade and tests are available today whereby practically every known function of the adrenal cortex may be evaluated separately. It would appear that clinical differences within the same disease process will be confirmed by finding unequal impairment of various functions in what grossly appears to be the same disease. With recent advances in the specific replacement therapy, survival of patients with Addison's disease has been improved greatly. It is apparent, however, that further improvement in mortality rate and in clinical conditions will depend upon the availability of 11-oxy-steroids for use in conjunction with a basic maintenance dose of desoxycorticosterone acetate. In the absence of active tuberculosis, such amplified replacement therapy should make it possible for patients with Addison's disease to lead a useful and independent existence.

References

1. THORN, G. W., S. S. DORRANCE, & E. DAY. 1942. Addison's disease: Evaluation of synthetic desoxycorticosterone acetate therapy in 158 patients. Ann. Int. Med. **16:** 1053.
2. ROWNTREE, L. G. & A. M. SNELL. 1931. *A Clinical Study of Addison's Disease.* Mayo Clinic Monographs. Saunders. Philadelphia.
3. ROBINSON, F. J., M. H. POWER, & E. J. KEPLER. 1941. Two new procedures to assist in the recognition and exclusion of Addison's disease. A preliminary report. Proc. Staff. Meet. Mayo Clinic. **16:** 577.
4. CUTLER, H. H., M. H. POWER, & R. M. WILDER. 1938. Concentrations of chloride, sodium, and potassium in urine and blood. Their diagnostic significance in adrenal insufficiency. J. A. M. A. **111:** 117.
5. DUNGER, R. 1910. Eine einfache Methode der Zaehlung der eosinophilen Leukozyten und der praktische Wert dieser Untersuchung. Münch. Med. Wochenschr. **57:** 1942.
6. LOEB, R. F. 1933. Effect of sodium chloride in treatment of patients with Addison's disease. Proc. Soc. Exp. Biol. & Med. **30:** 808.
7. HARROP, G. A., A. WEINSTEIN, L. J. SOFFER, & J. H. TRESCHER. 1933. Diagnosis and treatment of Addison's disease. J. A. M. A. **100:** 1850.
8. FORSHAM, P. H., G. W. THORN, G. E. BERGNER, & K. EMERSON, JR. 1946. Metabolic changes induced by synthetic 11-dehydrocorticosterone acetate. Am. J. Med. **1:** 105.
9. PERERA, G. A., D. W. BLOOD, & K. H. REINHOLD. 1946. 11-Dehydrocorticosterone. Its effects on hypoadrenalism in man. Am. J. Med. **1:** 135.

CUSHING'S DISEASE: A PRIMARY DISORDER OF THE ADRENAL CORTICES?

By EDWIN J. KEPLER*
Division of Medicine, Mayo Clinic, Rochester, Minnesota

Cushing's disease was selected as a subject with the hope that it might appeal to the experimentalist as well as to the clinician who is interested in endocrinology. Some of the data previously have appeared in print. More will be reported elsewhere. At the present time, repetition of this sort seems to be unavoidable.

Literature pertaining to Cushing's syndrome has accumulated with a rapidity suggestive of a geometric progression, whereas my ability to read it proceeds arithmetically. Consequently, I shall review comparatively little of it. Instead, I shall restrict myself to a summary of impressions that I have gained from reading a portion of it. This portion, I hope, has been representative.

By necessity, I shall draw heavily from my own personal experience, which, because of my position, has been relatively extensive. It is necessary to use the word "relative" because Cushing's disease occurs infrequently, and consequently no one person ever has the opportunity to study a large series of cases. Its incidence is not known. In the larger cities, patients afflicted with it occasionally may be seen on the streets and in public places. In smaller communities, it is distinctly a rarity. For example, in the twenty-odd years that I have lived in Rochester, Minnesota, a town of about 35,000 people, to my knowledge not a single case has appeared locally. Had any such cases occurred, almost certainly the patient would have consulted the Mayo Clinic, since few patients in the city or its immediate environs seek medical advice elsewhere, once they become seriously ill.

To conclude these introductory remarks, I must emphasize the fact that few, if any, of the basic concepts that I have pertaining to the subject are original. They represent merely the end result of the intellectual contacts which I have had with immediate associates and scientific friends elsewhere.

It was in 1932 that Cushing[1, 2] published his original articles on basophilic tumors of the hypophysis. In them he called attention to an old but unusual "polyglandular" clinical picture, which, hereafter, I shall refer to as Cushing's syndrome.† Its salient features are: a distinctive habitus characterized by wasting of muscle and by obesity or abnormal distribution of fat, the combination of which makes the trunk, face, and neck appear obese and the extremities thin; hirsutism; hypertension; osteoporosis; diabetes, either latent or frank; ecchymosis; and amenorrhea or impotence. Usually, there is a pad of fat over the cervicothoracic portion of the spinal column. The eyelids and corners of the mouth droop, and, when the face is viewed squarely from the front, the ears may be partially or totally concealed by the fullness of the face. Almost always there is an easily demonstrable muscular weakness, sometimes of a degree seen in moderately advanced muscular dystrophy or atrophy, so that in stepping up ordinary stairs the patient may have to use his hands to lift his feet. Acne of varying intensity is fairly common. Generally, the skin is thin and there are distinctive purplish striations. Polyuria occurs fairly frequently. Sometimes there is erythremia; nearly always there is lymphopenia. The concentration of the plasma potassium is reduced more frequently than has usually been reported, and occasionally a full-blown hypochloremic alkalosis is present, with or without elevation of plasma sodium. The amount of urinary 17-ketosteroids may be normal or increased moderately or greatly, depending on the pathologic processes responsible for the clinical picture. In the cases studied, assay of the urine for corticosteroids or chemical analysis for these substances, by the method developed by Talbot and his associates,[3] has disclosed increased values.[4] What combination of symptoms constitutes a *sine qua non* for making the diagnosis is a matter of opinion.

---

* Died October 19, 1947.

† The term "Cushing's syndrome" will be used to designate the clinical picture described by Cushing. The term "Cushing's disease" will be used to designate all cases presenting this clinical picture, except those associated with an adrenal cortical tumor. In other publications, I have employed the term to include only those cases in which the clinical picture was associated with a basophilic tumor of the anterior pituitary body.

Cushing, in a series of cases, found at necropsy that the clinical picture just mentioned was associated with small basophilic tumors of the pituitary body. In other instances, he made an accurate anatomic diagnosis before death. Furthermore, he predicted correctly that if the pituitary body were re-examined in certain cases which had been reported in the literature, a basophilic adenoma could be demonstrated. He attributed the clinical picture to the secretory activity of these tumors. His reasoning is refreshing in its simplicity. Essentially, it was as follows: Three types of cells are found in the anterior pituitary body; namely, chromophobic cells which are nonsecretory, and eosinophilic and basophilic cells which secrete hormones. Any one of these three cell types may give rise to its own peculiar adenomatous formations. The chromophobe tumors, consisting of nonfunctioning cells, are destructive nonfunctioning lesions. Adenomas arising from the eosinophilic cells are functionally active and produce the clinical picture known as acromegaly. Adenomas arising from basophilic elements, theoretically, therefore, also should have functional activity. The secretory products of such basophilic adenomas in turn would affect the function of other members of the endocrine system, particularly the gonads, the adrenal cortices, and other loci as well. As a result, "a polyglandular syndrome hitherto supposed to be of cortico-adrenal origin might be brought about."

Immediately after Cushing's articles were published, further examples of the syndrome were reported and data on small series of cases began to appear in the literature.* Simultaneously, two main schools of thought developed. Members of the first school emphasized the pathogenic importance of the pituitary body. Members of the second, exemplified, for example, by Kepler and his associates,[14] Bauer,[15] Kraus,[16] Anderson, Haymaker, and Joseph,[17–19] Albright,[20] and others listed by Luft,[13] for reasons to be shown subsequently in this paper, stressed the pathogenic potentialities of the adrenal cortices. Evidence accumulated to support each of these divergent schools of thought. Some of it is based on observations made by clinicians and pathologists. The remainder consists of studies conducted on laboratory animals by workers engaged primarily in fundamental research.

---

* Reviews will be found in articles by Freyberg and his associates,[5] Kessel,[6] Bland and Goldstein,[7] Tesseraux,[8] Haymaker and Anderson,[9] Eisenhardt and Thompson,[10] Kepler,[11] Thompson and Eisenhardt,[12] and Luft.[13]

In the material that follows, I shall review a few of the older observations, examine critically conclusions that have been drawn from them, and then present clinical data recently acquired by my associates and myself.

Evidence from Pathological Material

Cushing's contention lost ground when it was shown that the small basophilic adenomas which he described could be found comparatively frequently when searched for routinely at necropsy.[21-23] Since the adenomas occurred so frequently and the clinical picture so rarely, it seemed entirely logical to conclude that the relationship of the adenoma to the clinical picture was fortuitous and not causative. This conclusion has always seemed faulty to me. Applying the same line of reasoning to adenomas of the ductless glands, one could conclude that adenomas of the islet cells of Langerhans never caused hyperinsulinism, that adenomas of the thyroid gland never caused hyperthyroidism, and that adenomas of the adrenal cortex never caused adrenogenitalism, since in all three instances the incidence of adenomas without clinical evidence of hyperfunction is greatly in excess of that of those with clinical evidence of hyperfunction. No clinical or surgical endocrinologist would be willing to accept this conclusion.

As Cushing anticipated, objections to his thesis were raised because the tumors he discovered were small, whereas the clinical disturbances that accompanied them seemed to be disproportionately great. Subsequently, however, it was demonstrated that not all of the tumors were small. In fact, some were large enough to erode the sella turcica and were associated with all the significant endrocrinological abnormalities that he had mentioned.[24-26] In addition, as he pointed out in his original articles, size *per se* cannot be regarded as a criterion of adenomatous functional capacity. This lack of correlation between size and physiological disturbances is particularly evident in the case of parathyroid tumors and pancreatic islet cell adenomas.

Cushing's general thesis was both strengthened and weakened by observations made by Crooke.[27, 28] These observations have been confirmed repeatedly.[12, 23, 29]* He examined the pituitary body in a series of cases of Cushing's syndrome and found, in each instance, the cytologic appearance of the basophilic cells altered in a peculiar and specific fashion. The cytoplasm was hyalinized, degranulated, and contained vacuoles. These changes were not found in the cells of the basophilic tumor, if one happened to be present, but were confined entirely to the cells in the non-tumorous portion of the gland. They were almost entirely limited to cases in which the clinical picture was that described by Cushing and occurred invariably in these instances, even when gross macroscopic lesions were found in other endocrine structures such as the adrenal cortices and the thymus. Crooke concluded that these microscopic alterations of the basophils were the pathological denominator common to all cases of Cushing's syndrome and that, therefore, they were the anatomic expression of the disturbed function of the basophilic cells which gave rise to the clinical picture. His concept obviously threw out the basophilic tumor as an etiologic factor, but still kept the anatomical and functional source of the disorder confined to the pituitary body and the basophilic cells. For reasons which have been published elsewhere,[31] this conclusion was not inevitable. The changes which he described can logically be regarded as a retrograde* or degenerative lesion, secondary to disordered function and hyperactive in character, of the adrenal cortices. Kraus,[16] in 1937, came to a similar conclusion.

---

* Kraus,[16] in 1937, and Severinghaus,[30] in 1938, commented on cells that were very similar to, if not identical with those described by Crooke.

In many cases of Cushing's syndrome, hyperplastic or neoplastic lesions of the adrenal cortex have been discovered either at operation or at necropsy. The exact incidence of pathological changes in the adrenal cortex has not been determined, but some idea of their frequency can be obtained from two series of cases that have been analyzed recently. The first series, containing 98 cases, was collected by Thompson and Eisenhardt.[12] In it, there were 22 cases of adrenal cortical tumor. The second series was reviewed by Gorsuch.[33] From the literature, he selected seventy-two fatal cases in which the clinical picture conformed closely to that described by Cushing. He tabulated the gross pathological lesions as follows:

Neither pituitary nor adrenal pathological change in 7 cases.
Basophilic pituitary tumor without adrenal pathology in 11 cases.
Miscellaneous pituitary tumors (not basophilic) in 6 cases.
Adrenal cortical adenoma in 8 cases.
Adrenal cortical carcinoma in 7 cases.
Adrenal cortical hyperplasia in 9 cases.
Pituitary and cortical lesions in 24 cases.

Crooke's changes were not mentioned, but, presumably, they were present in most of the cases. Also, adrenal cortical hyperplasia, rightly or wrongly, was excluded if the smaller of the two adrenal glands did not weigh 10 gm. or more. It is evident that, in less than 22 per cent of Gorsuch's cases, pituitary lesions only were present, in one third, adrenal lesions only, and in another third, lesions were present in both organs. Fifteen cases of adrenal cortical carcinoma were excluded from this series of seventy-two fatal cases because some of the patients recovered and because the diagnosis was made by surgical exploration. In an appreciable number of instances of Cushing's syndrome, the adrenal cortices have been described as being normal in size and microscopic appearance, and in one case described by Freyberg and his associates[5] both adrenal cortices were thought to be "hypoplastic."† Adherents to the adrenal school of thought naturally have been impressed by this high incidence of adrenal cortical lesions and regard the so-called normal adrenal glands that are encountered as being examples of the shortcomings of histologic technique or interpretation.

---

* Kraus[32] recently has reviewed elsewhere the various cytologic changes that occur in the anterior pituitary consequent to primary disease.

† These authors felt that the histologic appearance of the cells did not necessarily signify decreased secretory activity.

Advocates of the pituitary theory accept the high incidence of adrenal cortical lesions and regard them as being the result of intense stimulation of the adrenal cortices by the abnormal anterior pituitary body. In the case of malignant adrenal cortical tumors, they naturally have to assume not only stimulation by the basophilic cells of the pituitary body, but also the existence of those specific and predisposing factors which are responsible for the development of malignancy in general. Unilateral malignant adrenal tumors, particularly when associated with Cushing's syndrome, have often been found to be accompanied by functional and anatomical contralateral cortical atrophy. It is particularly difficult to adapt the pituitary theory to this unpleasant fact.* The theory implies that a pituitary trophic agent could stimulate one adrenal cortex to a point where neoplastic changes could occur and simultaneously not only fail to stimulate the other adrenal cortex, but actually permit it to atrophy. Of course, one might argue that the contralateral gland was always atrophic and that compensatory hyperplasia was followed by neoplasia of the opposite side. Clinically, this explanation is not satisfactory, because the atrophic contralateral gland does not seem to be permanently incapacitated. Once the functioning adrenal neoplasm is removed, the contralateral atrophic gland rather promptly begins to function and presumably regenerates.

A few isolated pathological observations have been made which are difficult to account for by either theory. In a small number of cases of Cushing's syndrome, malignant lesions of the pancreas or of the thymus have been discovered at necropsy.[34, 35] In most of these cases, the adrenal cortices have been found to be hyperplastic.

I will consider the thymic cancer first. Because it happens to have been present, many authors have tacitly concluded that it must be a functioning tumor, which, in some mysterious fashion, produced the clinical picture. I say mysterious because, to my knowledge, there is no good evidence to indicate that the thymus secretes any hormonal products, and no evidence whatsoever that any hormones it might secrete could possibly produce the symptoms of Cushing's syndrome. For the time being, at least, the thymic tumors, therefore, can best be regarded as coincidental or secondary phenomena. Personally, I think they are probably secondary and not coincidental. Thymic tissue in cases of Cushing's syndrome is probably very active tissue, possibly because of the action of corticosteroids of the Compound E (11-dehydro-17-hydroxycorticosterone) type on lymphoid tissue. White and Dougherty[36, 37] have shown that the administration of Compound E caused involution of the thymus and lymph nodes with liberation of gamma globulins and immune bodies. I suspect, but have no proof, that if overproduction of steroids like Compound E was continuous, thymic involution would be accompanied by regenerative hyperplasia.† (If this were not the case, all the lymphoid tissue of the body would soon be exhausted).

---

* However, it is easy to incorporate the fact in the adrenal theory. Theoretically, one hyperfunctioning cortex might depress the output of pituitary adrenotropin to such a degree that atrophy of the contralateral adrenal gland would result.

† Three months after this presentation was made we found regenerative hyperplasia at necropsy in one case. The patient died very shortly after admission to the clinic because of hypertension and heart failure. The only therapy employed consisted of the usual measures used in cases of congestive failure. The thymus was studied by Dr. Hal Downey.

Under such circumstances, carcinomatous changes in the thymus might be expected to occur, since carcinomatous changes seem to be particularly prone to develop in tissues in which breakdown and repair are accelerated. Several examples of this general phenomenon might be presented, but two will suffice. Gastric carcinoma not infrequently develops in the atrophic gastric mucosa of the patient who has pernicious anemia.[38] Carcinoma of the colon is a complication which occurs frequently in cases of ulcerative colitis.[39] In the case of Cushing's syndrome associated with carcinoma of the pancreas, one can only suspect that the pancreatic cells also may be in this state of flux. Irrespective of this point, there seems to be little doubt that the pancreas of the patient who has this syndrome is a particularly vulnerable organ, susceptible to cyst formation, abscess, fat necrosis, and carcinoma. Personally, I have seen examples of all these pancreatic complications. In a small series of cases reported by Mellgren,[40] although he did not call attention to them, there was a relatively high incidence of pancreatic complications.

Recently, Heinbecker[41] has described degenerative lesions in the paraventricular nuclei in cases of Cushing's disease without adrenal tumor. These changes, he concluded, were of pathogenic significance. He postulated that, when these nuclei degenerated, the pituitary body was released from hypothalamic control and that, as a consequence, the Crooke's cells* would hyperfunction. This conception, it will be noted, is essentially a modification of the pituitary theory.

Finally, there remain a few disconcerting pathological observations that occasionally have been encountered. For example, Reforzo-Membrives described, in a personal communication, a case in which neither gross nor microscopic abnormalities could be discovered in either the pituitary body or the adrenal cortices. Crooke's changes, although carefully searched for, could not be found. Thompson and Eisenhardt[12] mentioned instances of Cushing's syndrome associated with chromophobe adenomas of the pituitary body. The case of Freyberg, in which a basophilic tumor of the pituitary body was associated with adrenal cortical hypoplasia, has been mentioned previously. For the time being, these and similar cases can best be regarded as inexplicable exceptions to the general rule.

Clinical Evidence

In considering the etiology of Cushing's disease, one must keep in mind two major clinical observations.

First, a significant number of patients apparently have either been cured of their symptoms or materially benefited by roentgen therapy to the pituitary body. In my experience, the number of patients benefited by this type of treatment has not been impressive. Nevertheless, when remissions have followed roentgen therapy, they have been rather dramatic. Other clinicians, particularly Luft,[13] have reported more impressive results. It seems only fair, therefore, to accept the evidence at its face value and assume that the cure or remission was induced by the roentgen therapy that was employed. Failures conceivably might occur because a basophilic tumor was not present, or, if one was present, it was roentgenologically insensitive, or because in the presence of Crooke's changes the disordered cellular pathological physiology was not amenable to roentgen therapy. Ignoring the failures and concentrating on the successes, one is tempted to regard favorable response to roentgen therapy as proof for the pituitary etiology of Cushing's disease. Regarded as a problem in pure logic, it is relatively easy to show that this conclusion need not be correct. The erroneous reasoning arises from the fact that a distinction is not made between an etiologic factor and a necessary condition.*

---

* For brevity, I have used this term to designate pituitary basophilic cells which have the abnormal cytological appearance described by Crooke.

To make this point clear, I am going to consider for a moment the etiology of malaria as it appeared a century ago. It was known then not only that the presence of swamps or stagnant water, temperature, and direction of prevailing winds had an important influence on the incidence of malaria, but also that, when some of these factors changed, the epidemiological characteristics of the disease were altered. With the evidence available at the time, it was highly logical, but nevertheless completely erroneous, to conclude that malaria was caused by stagnant water, for example.

In a similar fashion, one can also regard the influence of the pituitary body in the pathogenesis of Cushing's syndrome as a condition necessary to its production rather than as a primary etiological factor. If this were the case and if the function of the pituitary body were altered by roentgen therapy, the syndrome might be expected to regress in some instances, just as the incidence of malaria might be greatly reduced when conditions necessary to the breeding of mosquitoes were eliminated. In short, then, favorable response to roentgen therapy alone does not necessarily establish the validity of the pituitary theory.

The second clinical observation of theoretical importance is the fact that Cushing's syndrome has been cured after the removal of an adrenal cortical tumor (FIGURES 1 and 2). This fact establishes the adrenal neoplasm as the immediate source of the endocrine symptoms. The evidence for this particular point is impressive. In one of the cases of Cushing's disease which I have studied, the patient at some time in the course of her illness had all of the symptoms mentioned by Cushing, as well as others, such as hypochloremic and hypokalemic alkalosis. Following removal of an adrenal cortical neoplasm, all of these symptoms disappeared. The tumor recurred and the symptoms did likewise. Removal of the recurrent tumor was followed by remission of symptoms. Finally, the recurrent tumor recurred and metastasized and once again the endocrine symptoms reappeared. Examples of this sort, however, do not prove that the disorder did not in reality begin as a specific type of basophilic dysfunction. In fact, a clinical course such as the one just described might be expected if the Crooke's cells (which, by the way, were present) initiated the disturbance and then continued to act after the tumor was removed. Cases of this type strongly suggest, but do not prove, that the hyperplastic adrenal cortices of Cushing's disease are

---

* It is rather remarkable how infrequently this distinction has been considered in current endocrinological theories regarding the relationship of the pituitary body to the pathogenesis of various diseases, such as hyperthyroidism and diabetes mellitus.

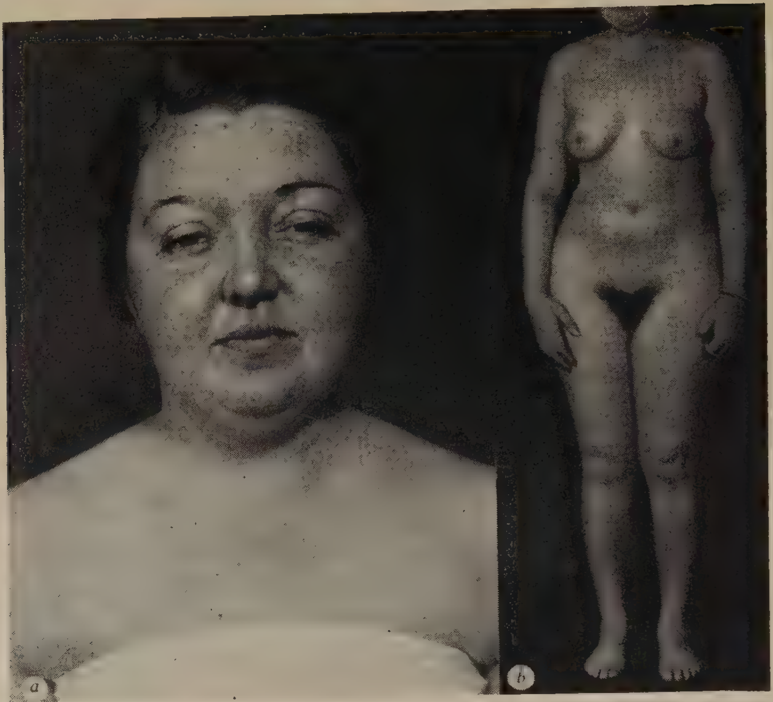


FIGURE 1. Cushing's syndrome associated with adrenal cortical tumor; *a* and *b*, appearance of patient before operation.

the immediate cause of the symptoms. One might argue that hyperfunctioning basophilic cells *per se*, without the mediation of the adrenal cortices, could produce clinical phenomena that are essentially the same as those produced by hyperfunctioning adrenal cortical cells. In this connection, the case of Freyberg carries some weight. Furthermore, diabetes produced experimentally, either by the administration of anterior pituitary preparations or by adrenal steroids or extracts, is an example of a single biological effect brought about by the action of two entirely different agents. Intuitively, however, the supposition that a distinctive, complex, and unusual clinical picture might be brought about by either one of two entirely different mechanisms is distasteful. Moreover, in the case of the malignant adrenal tumor previously mentioned, if the Crooke's cells were the immediate cause of any of the endocrine symptoms, the remission that followed the removal of the tumor should have been incomplete, or should not have occurred at all.

In his Harvey Lecture, Albright[20] summarized clearly and succinctly what I have called the adrenal theory:

> "*Major Premises*
>
> A. Patients with Cushing's Syndrome presumably have some common denominator in the etiology, as it would be unlikely that two entirely unrelated disorders would produce such a combination of clinical findings.
>
> B. Patients with cancer of the adrenals and Cushing's Syndrome presumably are suffering from an hyperadrenocorticism of some kind; *ergo* all patients with Cushing's Syndrome are suffering from an hyperadrenocorticism."

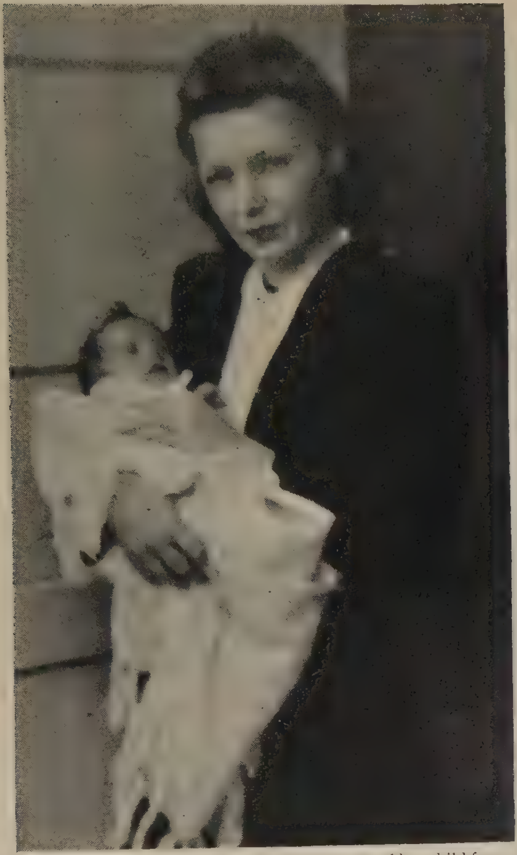


FIGURE 2. (Same patient as FIGURE 1.) Appearance of patient and her child four years after the removal of a benign encapsulated tumor (4 cm. in diameter, 9.0 gm. in weight) of the right adrenal gland. The left adrenal gland was examined also. Grossly, it appeared to be atrophic. A tiny portion was removed and this portion appeared to be atrophic on histologic examination. Eight years after operation the patient wrote that she had two children and considered herself to be well.

The logical consequences of the second of these two premises are far-reaching. I shall express them in a quasi-mathematical manner.

A. Assume that the abnormal basophilic cells in the pituitary body (either the Crooke's cells or the basophilic adenomas) are responsible for the pathogenesis of Cushing's syndrome.

B. Since none of the symptoms of the syndrome need be attributed directly to abnormal basophilic function, and since all of the symptoms can be attributed to adrenal cortical hyperfunction, then, the part played by the basophilic cells in the pathogenesis of the syndrome probably is indirect, remote, and restricted to the production of only one type of hormonal agent, presumably adrenotrophic in character. (If more than one basophilic agent were produced, endocrine symptoms would persist after the removal of a hyperfunctioning adrenal cortical lesion.)

C. If the abnormal Crooke's basophilic cells produce only one type of hormonal agent, normal basophilic cells *a priori* might produce only one type of hormonal agent.

D. If normal basophilic cells produce only one type of hormonal agent, all other types of anterior pituitary hormonal agents are produced by the nonbasophilic cells.

E. Indirect experimental, clinical, and pathological evidence strongly suggests that basophilic cells are not restricted to the production of one type of hormonal agent. Therefore, *D* becomes untenable; or at least unlikely.

F. If *D* is fallacious, *A* is reduced, if not to an absurdity, at least to an improbability. In other words, neither the Crooke's cells nor the basophilic adenomatous cells play any part in the pathogenesis of the syndrome.

Another deduction follows. If the basophilic adenomas play no part in the pathogenesis of the syndrome, they may, like the Crooke's cells, be regarded as a retrograde phenomenon. If this were the case, a sequence of events such as the following might account for their presence. For some unknown reasons, the adrenal cortex begins to hyperfunction. The adrenal steroids discharged from the cortex have an adverse or even lethal action on the basophilic cells of the pituitary body, which then acquire the histologic appearance described by Crooke. As a compensatory phenomenon, adenomatous basophilic hyperplasia results, analogous to the nodular adenomatous-like hyperplasia which occurs when the liver is damaged experimentally by toxic agents.

It should be noted that the entire argument rests on a major premise which appears to be reasonably probable, but which has not been proved, namely: in all cases irrespective of the remote cause of the syndrome, the endocrine symptoms result from some sort of adrenal cortical hyperfunction. It should also be noted that I have disregarded one other possibility because, *a priori*, it seems unlikely, namely: in Cushing's syndrome for some unknown reason, pituitary basophilic hyperfunction is dissociated in a peculiar and specific fashion so that the abnormal cells make only one of several possible agents in excessive amounts. Eosinophilic tumors almost certainly produce several trophic agents. Reasoning by analogy, it seems unlikely that functioning basophilic tumors should be restricted to the production of only one trophic principle if the normal basophilic cells have polyhormonal potentialities.

Experimental Evidence

The experimentalist has contributed comparatively little that bears on the immediate problems that pertain to the etiology of Cushing's syndrome. So far as is known, neither domestic nor experimental animals are subject to Cushing's syndrome, and, to my knowledge, in contrast to acromegaly and gigantism, nothing comparable to Cushing's syndrome has ever been produced experimentally. Most of the efforts of the experimentalists have been directed toward the study of the effects that follow the administration of specific pituitary principles or various adrenal steroids. In these studies, isolated symptoms of the disorder have been produced, particularly if adrenal steroids were administered. From this type of investigation, it is possible to understand how many of the symptoms of Cushing's syndrome might be produced. For a detailed analysis of the mechanisms by which adrenal cortical hyperfunction might produce the symptoms I have mentioned earlier, I refer the reader to Kenyon's[42] review in "Surgery," Albright's Harvey Lecture,[20] and the manuscript which my associates and I prepared for the 1946 Laurentian Hormone Conference.[43] Very little of this experimental work, however, throws any light on the problem which I am discussing, namely: Is Cushing's syndrome a primary disorder of the adrenal cortices?

Two types of experimental study seem to bear directly on my subject. The first has to do with the experimental production of Crooke's changes. Creditable cytological changes comparable to those described by Crooke have been produced by Severinghaus and Thompson[44] by the administration of pituitary extracts. Here it is possible that the pituitary extract stimulated the adrenal cortex to hyperfunction and, by so doing, caused retrograde changes, of the type described by Crooke, in the basophilic cells of the pituitary body. The second pertains to the experimental production of anterior pituitary tumors. Chromophobe tumors of the anterior pituitary body have been produced experimentally by the administration of large amounts of estrogenic substances.[45, 46] If chromophobe tumors can be produced by steroids of this type, it is not unreasonable to think that in suitable subjects basophilic tumors might be induced by the administration of adrenal or other steroids.

Résumé of the Theories Regarding the Pathogenesis of Cushing's Syndrome

In TABLE 1, I have attempted to outline the more important theories regarding the pathogenesis of Cushing's syndrome. None of these theories has been established conclusively, nor has any one of them been definitely refuted. For the time being, I regard theories 4 and 5 as being the most probable, because, to me, they conform with the greatest amount of factual material obtained from clinical, pathological, and experimental sources.

In my discussion thus far, I have used the words "theory" and "assumption" frequently. This was done deliberately, because to date every form of therapy that has been advocated has been based on some underlying theory or assumption or both. As long as this state of affairs continues, theories and assumptions will have more than an academic interest and importance.

My concluding remarks will be devoted to a description of the consequences that followed the practical application of one theory and to some of these consequences which appeared somewhat unexpectedly and proved to be of considerable scientific interest.

*Recently Acquired Clinical Data**

The lack of any specific medical treatment, the uncertain response to roentgen therapy to the pituitary body, and the miserable prognosis that confronts many of these patients led Dr. Walters and me a number of years ago to

---

* This material was presented in September, 1946, at the Laurentian Hormone Conference.

TABLE 1
THEORIES REGARDING THE PATHOGENESIS OF CUSHING'S SYNDROME

Theory	Pathological changes induced by unknown remote cause		Pathological changes involved in the immediate cause of symptoms		Associated pathological changes		
	Organ	Pathological change	Organ	Pathological Change	Organ	Changes	Explanation for
1	Anterior pituitary body	Crooke's cells or basophilic adenoma or both	Anterior pituitary body	Crooke's cells or basophilic adenoma or both	None		
2	Anterior pituitary body	Crooke's cells or basophilic adenoma or both	Adrenal cortex	Hyperplasia or neoplasia	Adrenal cortex	Hyperplasia or neoplasia	Stimulated by pituitary basophilic hyperfunction
3	Anterior pituitary body	Crooke's cells or basophilic adenoma or both	Adrenal cortex	Hyperplasia	Adrenal cortex	Hyperplasia	
			Anterior pituitary body	Crooke's cells or basophilic adenoma or both			
4	Adrenal cortex	Bilateral hyperplasia	Adrenal cortex	Bilateral hyperplasia	Anterior pituitary body	Crooke's cells	(1) Retrograde from adrenal cortex
						Basophilic adenoma or both	(2) Adenomatous regeneration
5	Adrenal cortex	Unilateral neoplasm	Adrenal cortex	Adrenal cortical neoplasm	Anterior pituitary body	Crooke's cells	Retrograde from adrenal cortex
					Adrenal cortex	Contralateral atrophy	Mediated by anterior pituitary body

attempt to treat a few patients with Cushing's disease surgically. Generally, less than half of each adrenal gland was removed. The therapeutic results were not impressive.

Subsequently, as it became apparent that the symptoms might well be an expression of adrenal cortical hyperfunction, my associates and I decided that if more adrenal tissue were removed the results might be better. For a time, we considered seriously the advisability of performing total adrenalectomy. Ultimately we decided, however, to resort to a less radical procedure, feeling that there was a reasonable chance that subtotal bilateral adrenalectomy might be equally as effective and at the same time be less hazardous to the patient.

In the three years prior to March, 1947, four patients were selected for this type of therapy. In three (cases 1, 2, and 4), all of one adrenal gland was removed and subsequently about two thirds of the other; in a fourth (case 3), about 60 per cent of each adrenal gland was removed.

The postoperative course in each instance was almost identical in character, and I shall describe it here in considerable detail. Two digressions are indicated.

The first digression concerns the patients. All four, of whom three were women and one was a man, had the classic Cushing's syndrome in its worst form. One patient, the man (case 1), had congestive heart failure and was edematous to the waist line. All had severe hypertension. In only one of the cases was the urinary excretion of 17-ketosteroids significantly increased. One patient (case 3), at the second operation, was found unexpectedly to have a low-grade adrenal carcinoma about 1 inch (2.5 cm.) in diameter. We have reasons to believe that this carcinoma may not have been hyperfunctioning.

The second digression has to do with the ordinary sequence of events which occurs when a hyperfunctioning adrenal tumor is removed on one side and the opposite gland is either atrophic or functionally inadequate. If the patient has not been properly prepared, there develops, during the first twenty-four hours or shortly thereafter, because of transient, contra-adrenal cortical atrophy, a shock-like condition which is often fatal. The temperature rises to hyperpyrexial levels and the clinical picture resembles fulminating adrenal cortical insufficiency. If, by virtue of treatment, the patient does not die, improvement generally proceeds rapidly, and at the end of a week or more, it is obvious that he will recover. Three months after the operation, many of the symptoms will have disappeared, and ultimately, provided that there has been no recurrence of the tumor, the diagnostic signs of the former malady will have regressed completely. If all adrenal cortical tissue is removed, as seemed to be true in the case of Bartels[47] of the Lahey Clinic, in which a nonfunctioning tumor was removed, presumably arising from a solitary adrenal gland, ordinary Addison's disease seems to follow. In his case, immediately after the operation the classic picture of postoperative adrenal insufficiency developed. With treatment the patient, a woman, recovered. When treatment was reduced or discontinued, adrenal insufficiency recurred. Soon, it became apparent that continuous replacement therapy would be necessary. At the end of three months she was deeply pigmented and had all the other characteristic signs and symptoms of Addison's disease.

Let us return then to the postoperative course in the four cases under discussion. With these an entirely different sequence of events occurred, a picture of which we shall try to present in a composite form. Immediately after partial removal of the second adrenal gland, nothing of any particular moment happened. Convalescence seemed to proceed uneventfully. Some time during the first week after operation or shortly thereafter, the patients complained of nausea. They lost their appetite and vomited. The anorexia became more and more intense, until finally they refused almost all food. Even the sight of food became repulsive. About the same time, abdominal pain and tenderness made their appearance. Gradually, the chemical composition of the blood became disordered. This disturbance generally was characterized, when the patient was not being treated with adrenocortical extract or electrolytes, by low concentrations of both sodium and potassium. The values for blood urea, and, as time went on, the concentration of blood calcium, slowly tended to rise and eventually attained levels seen in hyperparathyroidism. Phosphate levels, however, were not depressed.

Little by little, the patients lost their "Cushingoid" appearance. The plethora faded and the skin became pallid. Striations disappeared, ecchymoses no longer occurred after venipuncture, and it became possible to take the blood pressure without the production of petechiae. Imperceptibly, the hirsutism of the women vanished, so that the extremities became almost hairless. Growth of axillary hair was retarded. In every case, the urinary excretion of 17-ketosteroids declined until it reached levels characteristic of Addison's disease. The blood pressure varied but, in each instance, hypertensive values were obtained on most days irrespective of treatment. In case 3, administration of sodium chloride and sodium bicarbonate was followed by a sharp rise in blood pressure. Differential blood counts disclosed a gradual increase of the percentage of lymphocytes.

One of the patients, the man in case 1, seemingly was cured. Prior to the operations, his condition was the worst of the group. Two months after his second operation he was able to leave the hospital. By this time his condition was beginning to improve. During the next nine months he was seen on two occasions and, at each visit, fairly complete examinations did not reveal abnormalities of any consequence (FIGURE 3). His blood pressure had returned to normal and there was no question about the fact that he had neither Cushing's syndrome nor Addison's disease. The chemical composition of the blood and the daily urinary excretion of ketosteroids (10.7 mg.) were normal. Libido and potency, which had completely disappeared, had returned. At his last examination, eleven months after his second operation, his only complaint was that his physical stamina were below par as measured by his former standards. Nevertheless, he fathered a child. Some data in this case are summarized in TABLE 2.

Two of the remaining three patients (cases 2 and 4) died after a protracted illness (forty-two and sixty-six days). Both had gross disease of the pancreas. In one (case 4), there was evidence of acute and chronic

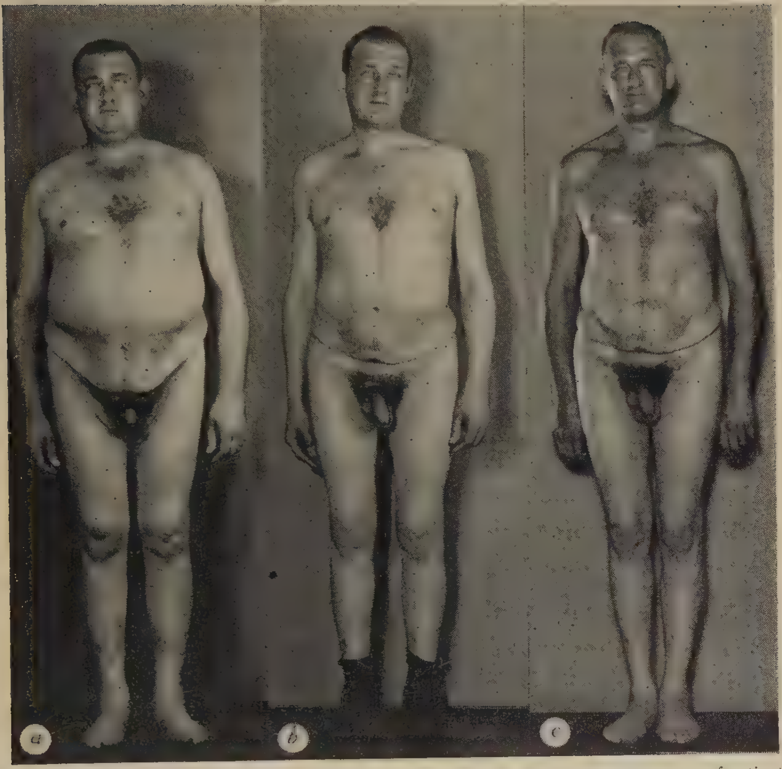


FIGURE 3. Cushing's syndrome not associated with adrenal cortical tumor: *a*, appearance of patient before bilateral subtotal adrenalectomy; *b*, appearance of patient 6 months later; *c*, appearance of patient about one year after operation.

hemorrhagic pancreatitis with cyst formation. This condition may have been present before her first operation, if any conclusions can be drawn from the medical history. In the other patient (case 2), fat necrosis was found in the tail of the pancreas. All of this is reminiscent of the "beef steak" pancreatitis that was described by Mann and Drips,[48] and at a later date by Code,[49] following adrenalectomy in dogs. Code has told me that these dogs refuse to eat and usually die, but, if they are fed by tube, some of them recover. Data in case 2 are given in TABLE 3.

In one patient (case 4) at necropsy, no adrenal cortical tissue could be identified. A basophilic adenoma about 2 mm. in diameter was found in the pituitary body, and, in the nonadenomatous portion of the gland, Crooke's changes were present in approximately 40 per cent of the basophilic cells. In the case of the other patient who died (case 2), traces of adrenal cortical tissue were identified. Unfortunately the pituitary body could not be examined.

The fourth patient (case 3) is alive four months (March, 1947) after removal of most of her adrenal cortical tissue and she is slowly improving. She no longer has any of the features of Cushing's syndrome that can be recognized on inspection. On the other hand, she does not have the appearance

TABLE 2

COURSE AFTER SUBTOTAL ADRENALECTOMY: CUSHING'S DISEASE—ADRENAL HYPERPLASIA (CASE 1, MAN, AGED TWENTY-NINE YEARS)

Date	Plasma						Blood pressure	Antecedent treatment					17-keto-steroids	Lympho-cytes
	mEq. per liter				Mg. per 100 cc.			Electrolyte			Hormonal			
	Na	Cl	CO_2	K	Ca	P		NaCl	Na citrate	KCl	Cortical extract	Testos-terone		
							mm. Hg						*mg. in 24 hr.*	%
7–17–45	139	99	34.4	4.0	10.4	3.5	175/135						30.9	12
8– 7–45	Left adrenalectomy						150/100					+		
8–28–45	136	100	28.0	4.7			145/85						15.6	
9– 7–45	Right subtotal adrenalectomy						170/110				+	+		
9–26–45	140	96	34.1	4.7	13.9		148/90				+	+		
10– 2–45		101	30.0		14.1	3.76	160/100	+	+	+				
10– 9–45	144	101	38.0	3.1	13.7	3.36	160/120	+	+	+			3.3	
11– 2–45	135	101	30.0	3.3	11.6	4.2	130/80							
	Remission													
2– 7–46	136	101	29.0	4.5	10.2	3.5	134/90						8.6	
8– 6–46	136	100	26.0	5.4	10.0	4.1	132/90						10.7	21

TABLE 3

COURSE AFTER SUBTOTAL ADRENALECTOMY: CUSHING'S DISEASE—ADRENAL HYPERPLASIA (CASE 2, WOMAN, AGED TWENTY-NINE YEARS)

Date	Plasma						Blood pressure	Antecedent treatment		17-keto-steroids	Lymphocytes
	mEq. per liter				Mg. per 100 cc.			Electrolyte	Cortical extract		
	Na	Cl	CO_2	K	Ca	P		KCl			
							mm. Hg			mg. in 24 hr.	%
11–13–45	135	103	26	4.2	9.1	3.3	170/110			1.2	6
11–23–45	Left adrenalectomy								+	2.9	
11–30–45	136	100	28	4.5	7.5	3.6	165/125				
3– 2–46	Right subtotal adrenalectomy						160/115		+		10
4– 2–46	135	93	31		11.8	2.9	150/100	+	+	1.4	
4– 8–46					13.4	2.8	168/145	+	+	0.3	
4–14–46	134	99	25.2	4.9			190/140	+	+		29
5– 1–46	136	100	23	2.6	10.3		170/110		+		
5– 7–46	137	110		5.0	8.9	4.4	160/120	+	+		

of patients who have Addison's disease. There is no pigmentation of the skin and repeated attempts to induce pigmentation by exposure to ultraviolet light have failed. The skin neither burned nor tanned. Comparatively early in her postoperative course, she complained of severe, continuous, upper abdominal pain. This finally disappeared after treatment with streptomycin was instituted, and, at the time, we suspected that she also had some type of pancreatitis. Her outstanding residual symptoms have been anorexia and profound muscular weakness. She still has mild hypertension and there is evidence of impaired renal function, although both of these seem to be improving slowly (TABLE 4).

In all four instances, it was difficult to make a distinction between those symptoms that were part and parcel of the postoperative disorder itself and those that might have been induced by treatment, either hormonal or with electrolytes. In the face of a decreased concentration of sodium in the plasma, we naturally were reluctant to discontinue the administration of cortical extract and sodium chloride, and, as a consequence, all four patients received such treatment in liberal amounts for varying periods after operation. In one patient (case 3), who had undergone partial removal of both adrenals, however, we decided to take the risk and discontinue all treatment. This was done for a period of about one month. During this month there was no appreciable change in the patient's clinical condition. The general level of the blood pressure fell but still persisted at levels that could be regarded as hypertensive. Azotemia persisted. The concentration of sodium in the blood remained low, while that of potassium slowly increased and finally reached low normal values. The excretion of 17-ketosteroids in the urine remained at Addisonian levels.

At the end of this period of study, testosterone propionate was administered in doses of 25 mg. three times a week. The patient's general condition immediately began to improve. Her appetite picked up, she became stronger, and the waxy pallor of the skin was replaced by a faint rubor. Thus far, this treatment has had no effect on the level of the blood urea or the blood pressure. Values obtained in the last chemical analysis the blood were as follows: sodium 135, mEq.; potassium, 4 mEq.; chloride, 104 mEq.; bicarbonate, 27 mEq. (all per liter); calcium, 12.4 mg.; phosphorus, 3.5 mg.; and urea, 80 mg. (all per 100 cc.).

From these four cases, the following deductions seem to be justified: (1) Most of the symptoms of Cushing's syndrome are contingent on the presence of the adrenal cortices. (2) It is equally clear that in the absence of the adrenal cortices most of the symptoms of Cushing's syndrome are not contingent either on the presence of a basophilic adenoma or of Crooke's changes. (3) The persistence of Crooke's changes in one case, in the absence of all demonstrable adrenal cortical tissue, casts some doubt on the thesis that Crooke's changes are manifestations of a retrograde or inhibitory action of the adrenal cortices on the anterior pituitary body. However, Crooke's changes may possibly be permanent changes. Furthermore, sufficient time (sixty-six days) may not have elapsed following removal of adrenal cortical tissue for Crooke's changes to disappear. (4) The

TABLE 4

COURSE AFTER SUBTOTAL ADRENALECTOMY: CUSHING'S DISEASE—ADRENAL HYPERPLASIA (CASE 3, WOMAN, AGED THIRTY-EIGHT YEARS)

Date, 1946	Plasma						Blood pressure	Antecedent treatment				17-keto-steroids	Lymphocytes
	mEq. per liter				Mg. per 100 cc.			Electrolyte			Cortical extract		
	Na	Cl	CO_2	K	Ca	P		NaCl	Na citrate	KCl			
							mm. Hg					*mg. in 24 hr.*	%
4–26	142	103	33	4.5	9.4	2.5	210/130					10.0	16
5–8	Partial left adrenalectomy											10.0	
5–11	137	96		4.3									
5–22	Partial right adrenalectomy, removal of cortical tumor							+			+	7.5	
5–27	138	100	—	2.7	8.9	3.2	190/120	+			+	1.8	
6–3	134	100	—	6.0	9.8	4.8		+		+	+		
6–10	131	95		3.8			180/120	+			+	0.6	21
6–26	129	92		4.9	10.8	4.6	120/98					0.8	34
7–8	135	100	26	3.4			130/70	+			+	0.7	
7–15	135	93	27	3.4			220/150	+	+			0.4	
7–26	130	91	29	3.9	13.9	4.5	120/80					0.2	35
8–19	135	104	27	4.1	12.4	3.5	180/105					0.5	45

hypertension of Cushing's syndrome does not depend entirely on the presence of the adrenal cortices. The adrenal cortices may be necessary for the original occurrence of this hypertension, but they do not seem to be necessary for the continuation after it once has been initiated. (5) In cases of Cushing's syndrome, removal of most of the adrenal cortical tissue may be followed by an unusual or at least atypical form of adrenal cortical insufficiency, the manifestations of which are different in many respects from those which are seen in cases of Addison's disease. (6) Subtotal adrenalectomy seems to be a hazardous procedure in cases of Cushing's syndrome. Its therapeutic utility has not been established, in spite of the fact that one patient seemingly has been cured of his disorder and another is getting better.

To return to the question implied in my title—"Is Cushing's disease a primary disorder of the adrenal cortex?"—the answer at this time is that I do not know; but it might well be.

Bibliography

1. Cushing, H. 1932. Basophil adenomas of pituitary body and their clinical manifestations (pituitary basophilism). Bull. Johns Hopkins Hosp. **50:** 137–195.
2. Cushing, Harvey. 1932. Further notes on pituitary basophilism. J. A. M. A. **99:** 281–284.
3. Talbot, N. B., A. H. Saltzman, R. L. Wixom, & J. K. Wolfe. 1945. The colorimetric assay of urinary corticosteroid-like substances. J. Biol. Chem. **160:** 535–546.
4. Venning, Eleanor H. & J. S. L. Browne. 1947. Excretion of glycogenic corticoids and of 17-ketosteroids in various endocrine and other disorders. J Clin. Endocrinol. **7:** 79–101.
5. Freyberg, R. H., P. S. Barker, L. H. Newburgh, & F. A. Coller. 1936. Pituitary basophilism (Cushing's syndrome); report of a verified case, with a discussion of the differential diagnosis and treatment. Arch. Int. Med. **58:** 187–212.
6. Kessel, F. K. 1936. Morbus Cushing. Ein Überblick über Klinik und Kasuistik des basophilen Hypophysenadenoms. Ergebn. inn. Med. & Kinderh. **50:** 620–678.
7. Bland, P. B. & Leopold Goldstein. 1937. Pituitary basophilism; a review of 42 verified cases, with a report of a personal case. Surg., Gynec. & Obst. **65:** 644–656.
8. Tesseraux, H. 1937. Zur Kasuistik und Pathologie der Cushingschen Krankheit. Endokrinologie **18:** 379–394.
9. Haymaker, W. & E. Anderson. 1938. Syndrome arising from hyperfunction of adrenal cortex: adrenogenital and Cushing's syndromes—review. Internat. Clin. **4:** 244–299.
10. Eisenhardt, L. & K. W. Thompson. 1939. A brief consideration of the present status of so-called pituitary basophilism; with a tabulation of verified cases. Yale J. Biol. & Med. **11:** 507–522.
11. Kepler, E. J. 1939. Tumors of the suprarenal cortex, basophilic tumors of the pituitary body, and allied diseases. In: Piersol, G. M.: *The Cyclopedia of Medicine, Surgery and Specialties*, 224–256. F. A. Davis Company. Philadelphia.
12. Thompson, K. W. & L. Eisenhardt. 1943. Further consideration of Cushing syndrome. J. Clin. Endocrinol. **3:** 445–452.
13. Luft, Rolf. 1944. A study on hirsutism, Cushing's syndrome and precocious puberty. Acta. med. Scand. (Suppl.) **149:** 1–119.
14. Kepler, E. J., R. L. J. Kennedy, A. C. Davis, W. Walters, & R. M. Wilder. 1934. Suprarenocortical syndrome and pituitary basophilism: presentation of three new cases. Proc. Staff. Meet., Mayo Clin. **9:** 169–178.
15. Bauer, Julius. 1936. Was ist Cushing'sche Krankheit? Schweiz. med. Wochenschr. **66:** 938–939.
16. Kraus, E. J. 1937. Morbus Cushing und basophiles Adenom. Klin. Wochenschr. **16:** 533–536.
17. Anderson, Evelyn, Webb Haymaker, & Michael Joseph. 1938. Hormone and electrolyte studies of patients with hyperadrenocortical syndrome (Cushing's syndrome). Endocrinology **23:** 398–402.

18. Anderson, Evelyn & Webb Haymaker. 1944. Cushing's syndrome. J. Nerv. & Ment. Dis. **99:** 511–520.
19. Anderson, Evelyn, & Webb Haymaker. 1938. Adrenal cortical hormone (cortin) in blood and urine of patients with Cushing's disease. Proc. Soc. Exp. Biol. & Med. **38:** 610–613.
20. Albright, Fuller. 1942–3. Cushing's syndrome: its pathological physiology, its relationship to the adreno-genital syndrome, and its connection with the problem of the reaction of the body to injurious agents ("alarm reaction" of Selye). Harvey Lectures. **38:** 123–186.
21. Costello, R. T. 1936. Subclinical adenoma of the pituitary gland. Am. J. Path. **12:** 205–215.
22. Susman, William. 1935. Adenomata of the pituitary, with special reference to pituitary basophilism of Cushing. Brit. J. Surg. **22:** 539–544.
23. Ecker, A. D. 1938. The hyaline change in the basophil cells of the pituitary body not associated with basophilism. Endocrinology **23:** 609–617.
24. Teachenor, F. R. 1935. Pituitary basophilism, with the report of a case. West. J. Surg. **43:** 127–133.
25. Page, A. P. M., L. V. Roberts, & J. H. Biggart. 1937. Cushing's syndrome in a mulatto; with a histological report. Lancet **2:** 625–627.
26. Furtado, Diogo, & Xavier Morato. 1939. Syndrome de Cushing; basophilisme hypophysaire. Presse méd. **2:** 1632–1634.
27. Crooke, A. C. 1935. A change in the basophil cells of the pituitary gland common to conditions which exhibit the syndrome attributed to basophil adenoma. J. Path. & Bact. **41:** 339–349.
28. Crooke, A. C. 1944. Quoted by N. G. B. McLetchie. Pituitary basophilism syndrome of Harvey Cushing. J. Endocrinol. **3:** 332–346.
29. Rasmussen, A. T. 1938. The proportions of the various subdivisions of the normal adult human hypophysis cerebri and the relative number of the different types of cells in *pars distalis*, with biometric evaluation of age and sex differences, and special consideration of basophilic invasion into the infundibular process. In: *Proceedings of the Association for Research in Nervous and Mental Diseases*: The pituitary gland; an investigation of the most recent advances (1936). **XVII:** 118–150. Williams & Wilkins, Baltimore.
30. Severinghaus, Aura E. 1936. The cytology of the pituitary gland. *Proceedings of the Association for Research in Nervous and Mental Diseases:* The Pituitary gland; an investigation of the most recent advances (1936). **XVII:** 69–117. Williams & Wilkins, Baltimore.
31. Kepler, E. J. 1945. Relationship of "Crooke's changes" in basophilic cells of anterior pituitary body to Cushing's syndrome (pituitary basophilism). J. Clin. Endocrinol. **5:** 70–75.
32. Kraus, J. E. 1945. Hyperplastic disease of the adenohypophysis. J. Clin. Endocrinol. **5:** 42–51.
33. Gorsuch, Melvin T. Personal communication.
34. Leyton, Otto, H. M. Turnbull, & A. B. Bratton. 1931. Primary cancer of the thymus with pluriglandular disturbance. J. Path. & Bact. **34** (2): 635–660.
35. Kepler, E. J. 1933. Symposium: polyglandular dyscrasias involving abnormalities of sexual characteristics; report of four cases. Proc. Staff. Meet., Mayo Clin. **8:** 102–107.
36. White, Abraham & T. F. Dougherty. 1945. The pituitary adrenotrophic hormone control of the rate of release of serum globulins from lymphoid tissue. Endocrinology **36:** 207–217.
37. Dougherty, T. F. & Abraham White. 1947. An evaluation of alterations produced in lymphoid tissue by pituitary-adrenal cortical secretion. J. Lab. & Clin. Med. **32:** 584–605.
38. Kaplan, H. S. & L. G. Rigler. 1947. Pernicious anemia and susceptibility to gastric neoplasms. J. Lab. & Clin. Med. **32:** 644–653.
39. Bargen, J. A. & W. G. Sauer. 1944. The association of chronic ulcerative colitis and carcinoma. Clinics **3:** 516–534.
40. Mellgren, Jan. 1945. The anterior pituitary in hyperfunction of the adrenal cortex; an anatomical study with special reference to *syndroma Morgagni* and notes on prostatic hypertrophy. Acta path. et microbiol. Scandinav. (Suppl.)) **60:** 1–177.
41. Heinbecker, Peter. 1944. The pathogenesis of Cushing's syndrome. Medicine **23:** 225–247.
42. Kenyon, A. T. 1944. Adrenal cortical tumors—physiologic considerations. Surgery **16:** 194–232.

43. KEPLER, E. J., R. G. SPRAGUE, H. L. MASON, & M. H. POWER. 1948. The pathologic physiology of adrenal cortical tumors and Cushing's syndrome. In: Recent Progress in Hormone Research **2:** 345–389.
44. SEVERINGHAUS, A. E. & K. W. THOMPSON. 1939. Cytological changes induced in the hypophysis by the prolonged administration of pituitary extract. Am. J. Path. **15:** 391–412.
45. SELYE, HANS. 1944. Experimental investigations concerning the role of the pituitary in tumorigenesis. Surgery **16:** 33–46.
46. GARDNER, W. U. 1944. Tumors in experimental animals receiving steroid hormones. Surgery **16:** 8–32.
47. BARTELS, ELMER. Personal communication.
48. MANN, F. C. & DELLA DRIPS. 1915. The relation of the adrenals to the pancreas. Arch. Int. Med. **16:** 681–692.
49. CODE, C. F. Personal communication.

PUBLICATIONS

OF

THE NEW YORK ACADEMY OF SCIENCES

(LYCEUM OF NATURAL HISTORY, 1817–1876)

(1) The ANNALS (octavo series), established in 1823, contain the scientific contributions and reports of researches, together with the records of meetings of the Academy. The articles which comprise each volume are printed separately, each in its own cover, and are distributed immediately upon publication. The price of the separate articles depends upon their length and the number of illustrations, and may be ascertained upon application to the Executive Director of the Academy.

(2) The TRANSACTIONS, re-established as Series II in 1938, contain extended abstracts of papers presented before the regular sectional meetings of the Academy and other matters of general interest to Members, and are published monthly from November to June, inclusive.

Current numbers of the ANNALS and TRANSACTIONS are sent free to all Members of the Academy desiring them.

(3) The SPECIAL PUBLICATIONS, established in 1939, are issued at irregular intervals as cloth-bound volumes. The price of each volume will be advertised at time of issue.

(4) The MEMOIRS (quarto series), established in 1895, are issued at irregular intervals. It is intended that each volume shall be devoted to monographs relating to some particular department of Science. Volume I, Part 1, is devoted to Astronomical Memoirs, Volume II to Zoological Memoirs. No more parts of the Memoirs have been published to date. The price is one dollar per part.

(5) The SCIENTIFIC SURVEY OF PUERTO RICO AND THE VIRGIN ISLANDS (octavo series), established in 1919, gives the detailed reports of the anthropological, botanical, geological, paleontological, zoological, and meteorological surveys of these islands.

Subscriptions and inquiries concerning current and back numbers of any of the publications of the Academy should be addressed to

EXECUTIVE DIRECTOR
The New York Academy of Sciences
Central Park West at Seventy-ninth Street
New York 24, N. Y.